8EHQ-0504-15578 MR 275157

[redacted]

February 12, 2004

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Environmental Protection Agency
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2004 MAY 10 AM 9:38

Attention: TSCA Section 8(e) Coordinator:

[redacted] hereby submits the attached documents pursuant to the TSCA Section 8(e). This is the summary and evaluation conducted by Dr. Gunter Oberdorster with results of inhalation toxicity test performed by [redacted] on carbon fiber (CAS#7440-44-0, manufactured by [redacted]. Since the raw material of the carbon fiber used the mesophase (anisotropic) pitch, the carbon fiber is classified into a mesophase pitch based carbon fiber. The form of the carbon fiber is a short fiber; the diameter was 1-2 micrometers.).

Sincerely,

8EHQ-04-15578

[redacted]

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Subchronic Carbon Fiber Inhalation Study in Rats

[redacted]

January 2004

Subchronic Carbon Fiber Inhalation Study in Rats

1. Fiber Sample

Since the raw material of the carbon fiber used the mesophase (anisotropic) pitch, the carbon fiber is classified into a mesophase pitch based carbon fiber. The form of this carbon fiber is a short fiber; the diameter was 1-2 micrometers.

2. Carbon fiber biopersistence

2-1. Design

2-1-1. Target Exposure Concentrations

The dose levels will be adjusted (if technically feasible) in according with the following criteria:

Group	Test Item	Exposure Concentrations
1	Filtered Air	-----
2	Carbon Fiber	15 mg/m3

Air Control Group (Group 1): Rats will be treated with filtered air under the same conditions as animals exposed to fibers.

2-1-2. Allocation

All animals will be allocated to lung sampling for possible lung burden evaluation.

Sacrifice Time Point	GROUP 1 Air Control	GROUP 2 CARBON FIBER
1 Day	1 – 5	16 – 22
3 Days		23 – 29
14 Days		30 – 36
30 Days	6 – 10	37 – 43
90 Days	11 – 15	44 – 50
Reserve animals	51 – 55	

2-2. Evaluation of biopersistence results

A five-day inhalation study in rats with carbon fibers was performed at RCC (Basel, Switzerland) in order to determine the biopersistence of the fibers retained in the lung.

The carbon fibers had a geometric mean length of 6.74 μm and a geometric mean diameter of 0.92 μm in the aerosol. Electron micrographic images showed that some of the fibers were hollow. The exposure protocol followed the guidelines in the EU for the measurement of fiber biopersistence. Exposure was for 5 days, 6 hours a day, at a concentration of 16 mg/m^3. The number of fibers longer than 20 μm was 92 fibers/cm^3 in the exposure atmosphere. Rats were exposed in nose only exposure tubes in order to minimize contamination of the fur. Groups of seven rats were sacrificed at days 1, 3, 15, 29 and 92 after exposure. The lungs were excised and weighed. After chemical digestion of the lungs, the fibrous and non-fibrous particles that had been retained in the lung at the different timepoints were collected on filters and their numbers were determined by scanning electron microscopy. Fibers were categorized according to their lengths and diameters, and results were expressed as number of fibers retained in the lung in the following categories: Total fibers; WHO fibers; fibers less than 5 μm long (<5 μm); fibers 15-20 μm long: fibers more than 20 μm long (> 20 μm); fibers more than 40 μm long (> 40 μm); and non-fibrous particles (< 1 μm; 1-3 μm; > 3 μm).

At each of the 5 sacrifice time points, the lungs of the seven rats were excised and stored frozen until digestion. The chemical digestion method was carefully evaluated and validated using lungs of control animals that were spiked with a known amount of carbon fibers. The fiber analysis was performed at GSA (Neuss, Germany) according to well established standardized counting protocols using scanning electron microscopy. No significant residues of lung tissues and no significant change of fiber surfaces was found after digestion, the retained fibers could well be characterized and counted.

Fiber retention in the lungs during the 92 postexposure periods shows a high dependency on fiber length. About 30% of the long fibers (> 20 μm) cleared very rapidly in this study over the first 2 weeks post exposure, and overall their retention halftime was 100 days. During this same time, the numbers of non-fibrous particles increased in the lungs, and also numbers of fibers < 5 μm in length were slightly higher on day 3, whereas fibers between 15 and 20 μm in length decreased continuously and disappeared from the lung with a retention halftime of 46 days. All fibers taken together were cleared with a retention halftime of < 60 days, which is consistent with effective alveolar macrophage mediated clearance. The fact that non fibrous particles, and to some degree

short fibers (< 5 μm), showed the above mentioned early apparent increase in the lung while the long fibers (> 20 μm) decreased, is consistent with a significant breakage of the long fibers during the clearance phase. The fragments of the broken long fibers then provide additional input into the short fiber and non-fibrous particle category, causing the observed increase over the first 2 weeks post exposure.

The most potent fiber size in terms of long term pathology, in particular with respect to a carcinogenic potential, are fibers longer than 20 μm. This well accepted concept of fiber toxicology is based on earlier pioneering studies which had shown that longer asbestos and glass fibers are the most potent ones for inducing lung tumors and mesothelioma. One reason for this is that fibers above a certain length can not be phagocytized by alveolar macrophages. These cells are present in the alveolar spaces of the lung and eliminate (clear) foreign materials effectively, provided this material is small enough to be taken up by these cells. Since the diameter of alveolar macrophages is between 12 (rats) to 20 (humans) μm, fibers longer than 20 μm are unlikely to be cleared by these cells and are therefore most likely to interact with target cells in the lungs.

In this study, breakage of carbon fibers appears to be important for the overall reduction in numbers of long fibers in the postexposure phase resulting in the elimination of the fiber category with the highest carcinogenic potential. The resulting shorter carbon fibers and non-fibrous carbon particles can be effectively cleared by alveolar macrophages. Indeed, the retention halftime of the non-fibrous particles in this study – after the initial increase – is ~60 days, and for the other fibers shorter tan 20 μm it is even less than 60 days. These values are within the range of 50–70 days, which are normal retention halftimes in rats to clear particles and short fibers of low toxicity contained in alveolar macrophages out of the lung.

The decrease of long fibers postexposure is statistically significant. Figure 1 shows the retention of the long carbon fibers (> 20 μm) as found in the study. In addition to the individual data points, the retention curve is plotted, showing a monoexponential decay. A compartmental analysis was performed to determine the statistical significance of the long fiber retention. For a single compartmental model, a statistically significant slope of 0.00647 (Standard Error: 0.00174) is calculated. The corresponding retention half time is 107 days for fibers longer than 20μm. There is also some evidence for a second compartment, but not enough data for a more precise.

According to the new testing guidelines of the European Union (EU) for synthetic vitreous fibers, long fibers (> 20 μm) of a new fiber product should be cleared from the rat lung with a halftime of 10 days or less in order to avoid being labeled as a potential carcinogenic fiber. The guidelines are specific for glassfibers, and there are as of yet no data to suggest that they should

apply to carbon fibers. Since elemental carbon is insoluble in the lung or in lung cells one would expect that long carbon fibers have a very long biopersistence (halftime of several hundred days). However, it appears that a significant breakage rate makes long carbon fibers smaller and accessible to normal lung clearance via phagocytic cells resulting in the halftime of 100-107 days. Thus, one could hypothesize that it is likely that no adverse effects from long carbon fibers will occur, unless very high lung burdens are present which overwhelm the alveolar macrophage clearance function due to overloading of the macrophages by large numbers of shorter carbon fibers.

This hypothesis is supported by results of an earlier short term carbon fiber study which showed that carbon fibers do indeed not cause more inflammation in the lung than a glass fiber that did not induce lung tumors in a previous chronic rat inhalation study . If the low inflammatory potency of this carbon fiber is confirmed in an inhalation study of longer duration, it would be strong evidence that these carbon fibers should not be categorized according to the EU rule with respect to the requirement of a 10 day retention halftime for long fibers.

In that earlier 5 day inhalation study with carbon fibers it was found that the inflammatory response in the lung induced by a similar lung burden of retained carbon fibers and MMVF-10 (a glass fiber which did not induce tumors in a chronic inhalation study) was the same: There was only an initial slight inflammatory response in both fiber groups which returned to baseline level by 10 days postexposure; this was in contrast to amosite asbestos which induced a significantly greater and lasting inflammatory response and which was used as a positive control in that earlier study. (Fig. 2) However, since this previous study was a short term exposure (5days inhalation) only and since results of a longer term subchronic inhalation exposure to carbon fibers are lacking, a final statement regarding the inflammatory or toxic response of carbon fibers can not be made at this point. It is conceivable, though, that the relationship between carbon fiber bio-persistence and lung toxicity is different for carbon fibers on the one hand and for glassfibers and asbestos on the other hand.

Thus, overall evaluation from the biopersistence study is that long carbon fibers are most likely cleared from the rat lung by breaking down to smaller fragments, which in turn can be cleared by normal clearance mechanisms through phagocytosis by alveolar macrophages. However, some long fibers (>20μm) may persist longer. Whether this fraction of the long carbon fibers, in combination with the shorter fiber fragments in the lung, can cause adverse effects needs to be determined in a subchronic inhalation study.

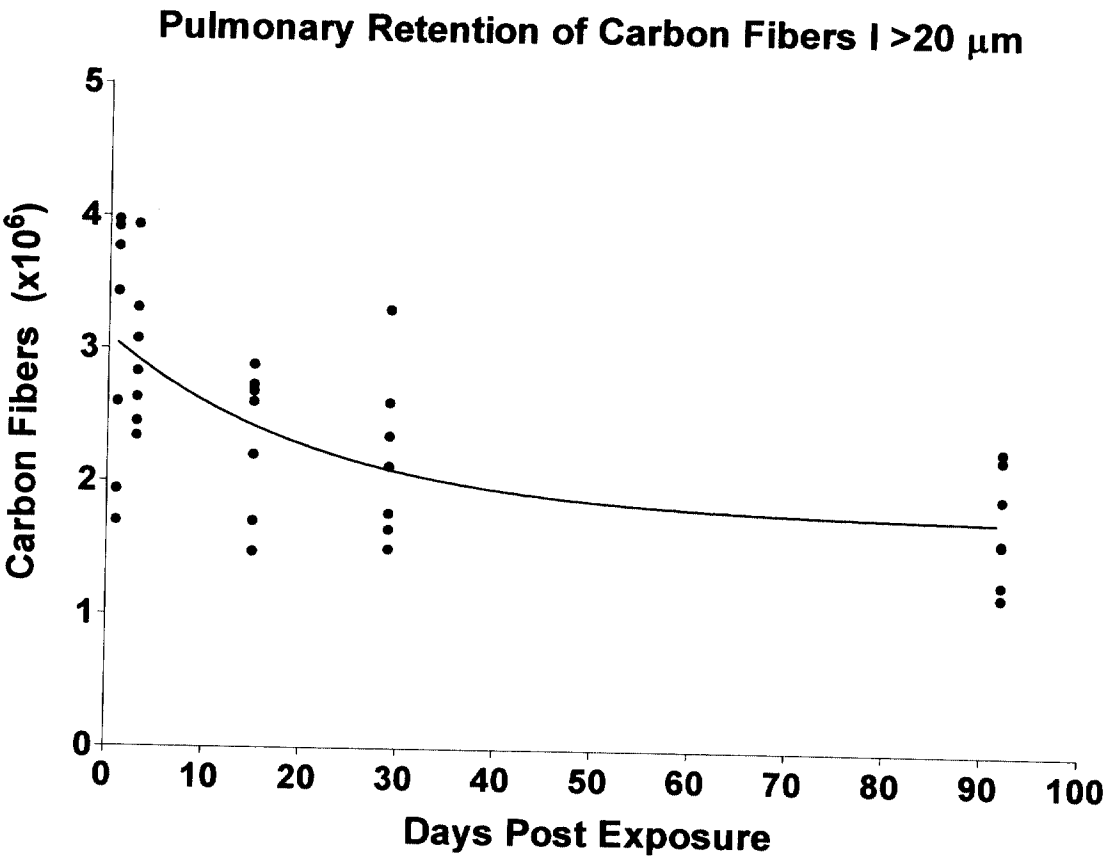

Figure 1. The retention of the long carbon fibers (> 20 μm)

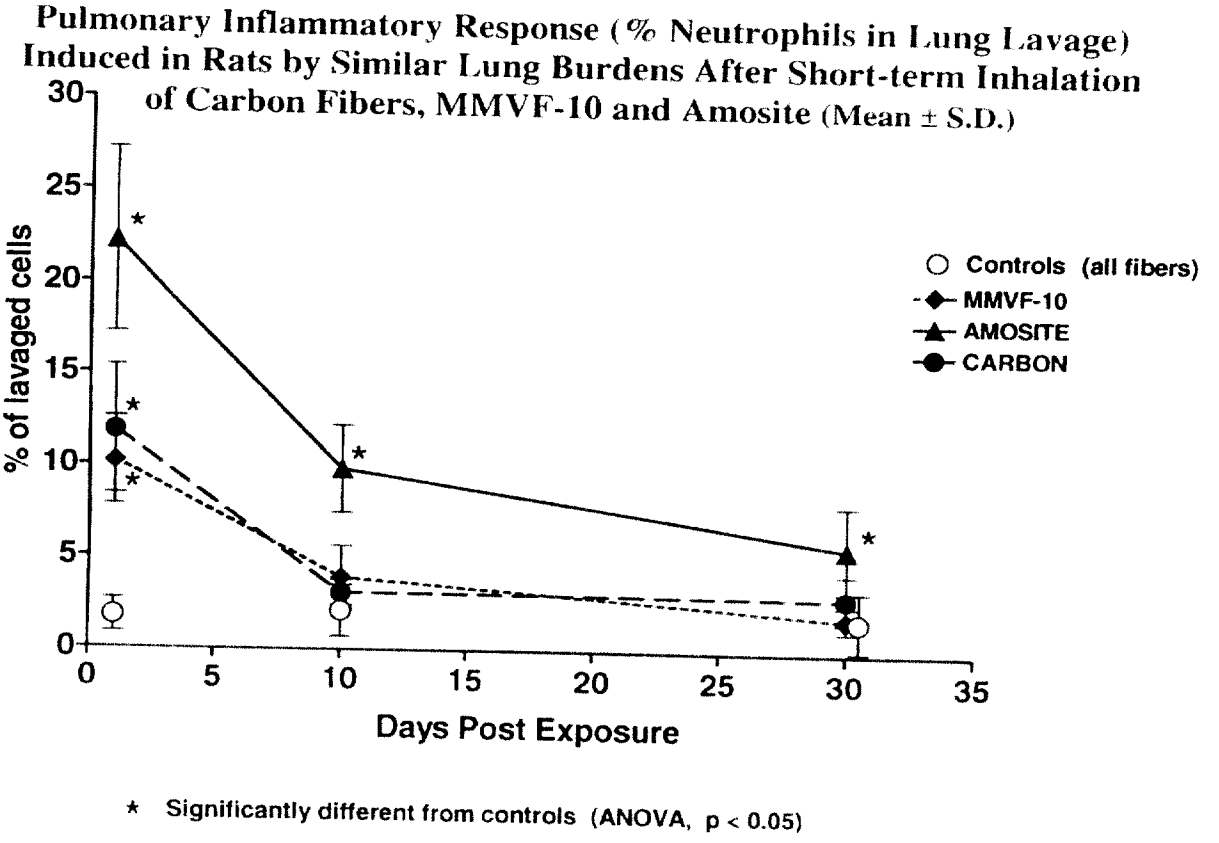

Figure 2. The pulmonary inflammatory response after short-term inhalation

3 Subchronic Carbon Fiber Inhalation Study

3-1. Design

A subchronic inhalation study in rats was performed for three dose groups of the carbon fiber sample. The 3 months exposure period was followed by a 3 months postexposure observation period. The rats were exposed 6 hours per day, 5 days per week for 3 months to a fiber aerosol concentration of 15, 50 and 150 fibers/ml (fiber length > 20µm). Within a 3-month observation period after end of 3 months exposure the rats were sacrificed at different intervals (see under Table1). The guideline of the European Commission ECB/TM 16(97) rev. 1 was used.

Table 1. List of investigations in the subchronic inhalation study
Exposure was terminated after 3 months; subgroups were kept for further 3 months for post-exposure investigations

Investigation	Test required after start of exposure [months]	Group	Number of animals per group for single investigation	Consecutive number of animals per group scheduled for investigation
Lung burden of test fibers	3 4 5* 6*	1-4	5 [a]	1-5 6-10*: 11-15*
Bronchoalveolar lavage	3 4 5 6	1-4	5	16-20: 21-25: 26-30
Histopathology/ BrdU	3 4 5 6	1-4	5	1-5 6-10 11-15
Reserve animals		1-4	2	31 32

* Lung burden analysis at 4 5 and 6 months optional

[a] The left lung lobe of animals scheduled for fiber burden analysis will be used for histopathology.

The study design is shown in Table 2

Table 2. Study design of the subchronic inhalation study, followed by a 3 months observation period

Exposure group	Group No	Fiber (Length>20µm) concentration (F/ml)	Gravimetric concentration* (mg/m^3)	Number of animals
Control (filtered air)	1	-	-	32
Carbon fibers low	2	~ 15	~ 2.5	32
Carbon fibers med	3	~ 50	~ 7.5	32
Carbon fibers high	4	~ 150	~ 25	32
Disease Surveillance				10
Total				138

* Estimation based on SEM analysis of pretest for aerosol generation

3-2. Method

3-2-1. Histopathology

Conventional histopathological examination including fibrosis scoring (H&E staining and Masson-Trichrome staining) was performed on the left lung lobes and trachea (H&E staining only) of animals listed in Table 2. The McConnell-Wagner fibrosis scoring system (McConnell et al. 1984) was used. Additionally the EPS evaluation system including scoring for collagen deposition at the bronchiolo-alveolar junction (Annex in ECB/TM/16(97) rev, 1) was used. A quantitative evaluation of fibrosis using a morphometric method according to the criteria from J. Davis, IOM, Edinburgh was also done.

The Wagner scoring system is from grade 1 to 8, and grade 4 correspond to evidence of early interstitial fibrosis. The EPS system includes grade 0 to 5 and grade 2 is corresponding to evidence of early interstitial fibrosis.

3-3. Results

3-3-1. Concentration and Fiber Size Distribution in the Aerosol

The aerosol concentrations were measured by SEM. The means and standard deviations of the fiber and particle concentrations in the aerosol samples of the three exposure chambers are summarized in Table 3. The size distribution of the aerosol samples is presented in Table 4 .

Table 3. Numerical and gravimetric concentration of fibers in the exposure chambers

Group		Numerical concentration [1/ml]				Mass concentration [mg/m³]		
		Fibers	WHO fibers	Fibers L>20µm	Particles	Estimation from SEM analysis* Fibers	Particles	Weighing of filter samples*
Carbon fiber Low	Mean	64 2	48 6	15 0	6 2	1 8	0 1	2 1
	SD	17 3	10 2	3 5	7.2	0 5	0 1	0 7
Carbon fiber Med	Mean	225 0	169 5	50 7	22 1	6 7	0 3	6 7
	SD	50 7	28 1	10 7	25 1	1 6	0 1	0 9
Carbon fiber High	Mean	693 6	531 1	158 3	73 7	21 5	0 9	20 6
	SD	115 2	85 4	27 2	66 1	3 9	0 5	2 5

SD Standard deviation

Table 4. Size distribution of aerosol samples (Mean values)

Group	Fiber length [µm]				Fiber diameter [µm]			
	Arithmetic		Geometric		Arithmetic		Geometric	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Fraction All fibers (L/D>3)								
Carbon fiber low	16.16	21.05	10.35	2.49	1.07	0.65	0.91	1.77
Carbon fiber med	16.28	20.32	10.37	2.51	1.09	0.67	0.92	1.78
Carbon fiber high	17.13	23.62	10.65	2.54	1.09	0.66	0.92	1.77
Fraction L>20µm								
Carbon fiber low	41.06	31.20	35.17	1.64	1.33	0.79	1.14	1.73
Carbon fiber med	41.14	28.62	35.41	1.63	1.38	0.83	1.19	1.72
Carbon fiber high	43.95	35.34	36.94	1.68	1.39	0.82	1.20	1.71
Fraction WHO								
Carbon fiber low	19.71	21.44	14.49	2.05	1.14	0.56	1.00	1.66
Carbon fiber med	19.94	21.27	14.57	2.06	1.17	0.58	1.04	1.66
Carbon fiber high	20.82	24.39	14.85	2.10	1.16	0.56	1.03	1.65

SD: Standard deviation

3-3-2. Lung Weights

The lung wet weights were determined of lungs taken for retention and BAL measurements. These data are given together with the terminal body weights in Table 5.

Table 5. Terminal body and lung wet weights

Group		Terminal body weight [g]		
		Postexposure interval [weeks]		
		1	8	15
Control	Mean	380.2	449.1	461.0
	SD	24.2	42.4	39.4
	N	10	10	11
Carbon fiber low	Mean	380.8	440.4	445.5
	SD	37.5	28.3	26.0
	N	10	10	12
Carbon fiber med	Mean	381.1	423.3	461.9
	SD	21.9	31.9	37.8
	N	10	10	12
Carbon fiber high	Mean	381.7	*407.1	451.3
	SD	31.9	33.0	28.3
	N	10	10	12
Group		Lung wet weight [g]		
		Postexposure interval [weeks]		
		1	8	15
Control	Mean	1.3139	1.3942	1.4615
	SD	0.0726	0.1473	0.1450
	N	10	10	11
Carbon fiber low	Mean	*1.4288	1.4724	1.5210
	SD	0.0660	0.0566	0.0806
	N	10	10	12
Carbon fiber med	Mean	***1.6060	**1.5737	**1.6788
	SD	0.0633	0.0942	0.1691
	N	10	10	12
Carbon fiber high	Mean	***1.7735	***1.6971	***1.8138
	SD	0.1176	0.1546	0.1600
	N	10	10	12

Statistics: Anova + Dunnett's tests (Two-Sided) * P<=5% ** P<=1% *** P<=0.1%
SD: Standard deviation N: Number of animals

3-3-3. Bronchoalveolar Lavage

During the recovery period bronchoalveolar lavages (BAL) were performed on 5 rats per group and sacrifice date (at the last sacrifice date 6 rats for the control group and 7 rats for treatment group were used). The following biochemical parameters were measured in the supernatant of the bronchoalveolar lavagate: lactic dehydrogenase (LDH), β-glucuronidase (ßGL) and total protein. The results are summarized in Table 6. The results of the differential cell count are presented in Table 7.

Table 6. Biochemical parameters in the BAL fluid

Group		1 week post exposure			8 weeks post exposure			15 weeks post exposure		
		LDH	ßGL	Protein	LDH	ßGL	Protein	LDH	ßGL	Protein
		U/l	U/l	mg/l	U/l	U/l	mg/l	U/l	U/l	mg/l
Control	Mean	38	0.2	99	34	0.3	105	33	0.2	103
	SD	8	0.1	10	3	0.1	10	10	0.1	16
	N	5	5	5	5	5	5	6	6	6
Carbon fiber low	Mean	67	0.3	**152	46	0.3	141	51	0.1	133
	SD	22	0.1	13	9	0.1	30	17	0.0	20
	N	5	5	5	5	5	5	7	7	7
Carbon fiber med	Mean	90	0.4	***182	66	0.3	***172	76	0.2	***179
	SD	39	0.1	34	11	0.1	19	55	0.1	24
	N	5	5	5	5	5	5	7	7	7
Carbon fiber high	Mean	***143	***0.7	***204	***122	0.3	***216	*109	0.3	***206
	SD	52	0.1	16	49	0.2	25	85	0.1	30
	N	5	5	5	5	5	5	7	7	7

Statistics: Anova + Dunnett's tests (Two-Sided) * P<=5%, ** P<=1%, *** P<=0.1%
SD: Standard deviation N: Number of animals

Table 7. Cell concentration and percentage of cells in the BAL fluid

Group		Postexposure interval [weeks]						
		1						
		Cell concentration [cells/ml]	Macropages [%]	Eosinophil PMNs [%]	Neutrophil PMNs [%]	Lymphocytes [%]	Cell Viability	Epithelial cells per 200 cells
Control	Mean	143250	98.8	0.0	0.8	0.5	99	3.2
	SD	41567	1.4	0.0	0.9	0.6	0	2.2
	N	5	5	5	5	5	5	5
Carbon fiber low	Mean	148500	95.2	0.1	3.0	1.8	100	4.0
	SD	6697	1.6	0.1	1.2	1.3	1	2.0
	N	5	5	5	5	5	5	5
Carbon fiber med	Mean	168750	***84.5	0.0	***8.7	**6.9	99	3.6
	SD	47145	2.5	0.0	0.5	2.4	1	2.1
	N	5	5	5	5	5	5	5
Carbon fiber high	Mean	162750	***80.4	0.0	***11.1	**8.6	99	4.8
	SD	33018	7.4	0.0	4.1	5.0	2	2.0
	N	5	5	5	5	5	5	5

Group		Postexposure interval [weeks]						
		8						
		Cell concentration [cells/ml]	Macropages [%]	Eosinophil PMNs [%]	Neutrophil PMNs [%]	Lymphocytes [%]	Cell Viability	Epithelial cells per 200 cells
Control	Mean	144000	99.5	0.0	0.3	0.3	98	3.0
	SD	13561	0.3	0.0	0.1	0.3	1	0.7
	N	5	5	5	5	5	5	5
Carbon fiber low	Mean	145000	97.3	0.1	1.5	1.2	99	3.4
	SD	27114	0.9	0.1	0.8	0.1	1	1.8
	N	5	5	5	5	5	5	5
Carbon fiber med	Mean	176250	**90.9	0.0	**5.4	*3.8	99	2.8
	SD	45303	4.7	0.0	4.0	3.1	2	2.5
	N	5	5	5	5	5	5	5
Carbon fiber high	Mean	*202500	**90.8	0.1	*5.3	*3.9	99	3.0
	SD	34267	4.1	0.1	2.3	1.9	1	1.6
	N	5	5	5	5	5	5	5

Statistics: Anova + Dunnett's tests (Two Sided) * P<=5% ** P<=1% *** P<=0.1%

SD: Standard deviation N: Number of animals

Table 7. (cont.) Cell concentration and percentage of cells in the BAL fluid

Group		Postexposure interval [weeks]						
		15						
		Cell concentration [cells/ml]	Macrophages [%]	Eosinophil PMNs [%]	Neutrophil PMNs [%]	Lymphocytes [%]	Cell Viability	Epithelial cells per 200 cells
Control	Mean	155000	98.8	0.0	0.5	0.8	99	7.3
	SD	22638	0.6	0.0	0.4	0.4	1	2.1
	N	6	6	6	6	6	6	6
Carbon fiber low	Mean	155000	96.9	0.0	2.1	1.0	99	3.6
	SD	39804	0.6	0.1	0.6	0.6	1	0.8
	N	7	7	7	7	7	7	7
Carbon fiber med	Mean	180536	**93.6	0.0	*3.6	**2.8	99	3.7
	SD	41975	1.8	0.0	1.4	1.3	1	1.6
	N	7	7	7	7	7	7	7
Carbon fiber high	Mean	194643	***90.8	0.0	***5.7	***3.5	99	3.1
	SD	48567	4.2	0.0	3.6	1.1	1	2.0
	N	7	7	7	7	7	7	7

Statistics: Anova + Dunnett's tests. (Two Sided) * P<=5% ** P<=1% *** P<=0.1%

SD: Standard deviation N: Number of animals

3-2-4. Time Course of Histopathological Findings

The time course of the main histopathological findings is given in Table 8.

Table 8. Summary of histopathological findings

Group	No of animals (out of 5) <with indicated lesion				No of animals (out of 5) <with indicated lesion				No of animals (out of 5) <with indicated lesion			
	Postexposure Interval				Postexposure Interval				Postexposure Interval			
	1 weeks				8 weeks				15 weeks			
	Score				Score				Score			
	very slight	slight	mode rate	total	very slight	slight	mode rate	total	very slight	slight	mode rate	total
Accumulation of fiber/particle-laden macrophages												
Control				0				0				0
Carbon fiber low	**5			**5	*4	1		**5	**5			**5
Carbon fiber med		**5		**5		**5		**5		**5		**5
Carbon fiber high		1	**4	**5		2	3	**5		1	**4	**5
Bronchiolo-alveolar hyperplasia												
Control				0				0				0
Carbon fiber low	*4	1		**5	3	2		**5	3	1		*4
Carbon fiber med		**5		**5		**5		**5	1	*4		**5
Carbon fiber high		**5		**5		**5		**5		**5		**5
Microgranulomas												
Control				0				0				0
Carbon fiber low	**5			**5	*4	1		**5	**5			**5
Carbon fiber med		**5		**5		**5		**5		**5		**5
Carbon fiber high		3	2	**5		3	2	**5		3	2	**5
Interstitial fibrosis												
Control				0				0				0
Carbon fiber low	**5			**5	**5			**5	3			3
Carbon fiber med	3	2		**5	*4	1		**5	*4	1		**5
Carbon fiber high		**5		**5		**5		**5		**5		**5

Significance of difference in a pairwise Fisher's test compared to control group *P<0.05 **P<0.01 ***P<0.001

3-3-5. Cell Proliferation Test

The results of the BrdU proliferation test are summarized in the Table 9.

Table 9. Proliferation index of lung tissue cells

Group	Unit length labelling index [%] of terminal bronchiolar epithelium at postexposure [positive cells per mm]								
	1 Week			8 Weeks			15 Weeks		
	Mean	SD	N	Mean	SD	N	Mean	SD	N
Control	2.14	1.47	5	3.29	2.00	5	2.30	1.25	5
Carbon fiber low	***9.77	1.88	5	6.73	3.09	5	*10.60	8.52	5
Carbon fiber med	***13.80	3.51	5	**12.70	4.14	5	**14.55	2.77	5
Carbon fiber high	***38.88	2.64	5	***15.11	3.92	5	***21.32	2.76	5
Group	Labelling index of lung parenchymal cells at postexposure [% positive cells]								
	1 Week			8 Weeks			15 Weeks		
	Mean	SD	N	Mean	SD	N	Mean	SD	N
Control	2.62	0.94	5	2.99	0.95	5	4.98	1.46	5
Carbon fiber low	3.31	0.46	5	4.03	1.03	5	4.86	1.16	5
Carbon fiber med	**4.25	0.43	5	*4.26	0.27	5	6.19	1.11	5
Carbon fiber high	***6.27	0.95	5	***5.39	0.50	5	***9.41	0.61	5
Group	Unit length labelling index [%] of pleural cells at postexposure [positive cells per cm]								
	1 Week			8 Weeks			15 Weeks		
	Mean	SD	N	Mean	SD	N	Mean	SD	N
Control	6.84	3.59	5	1.77	1.88	5	1.98	0.72	5
Carbon fiber low	7.63	3.98	5	3.76	0.81	5	2.95	1.56	5
Carbon fiber med	10.49	2.55	5	10.83	15.57	5	6.43	5.95	5
Carbon fiber high	*33.06	26.27	5	3.73	2.91	5	3.69	2.34	5

Statistics: Anova + Dunnett's tests (Two-Sided) * P<=5% ; ** P<=1% ; *** P<=0.1%
SD: Standard deviation N: Number of animals

3-4. Evaluation of subchronic results

---- Comparison of Carbon Fiber (Mesophase Pitch based Carbon Fiber) and MMVF-21 (Stonewool, Not carcinogenic in Rats), E-Glass (Microfibers, Durable Glass Fiber, carcinogenic in Rats) ----

The following paragraphs summarize the significant findings in terms of differences between carbon fiber and two other vitreous fibers which had previously been tested by the Fraunhofer Institute according to the same protocol. In my interim evaluation of this study, I had pointed out that the results of this carbon fiber subchronic rat inhalation study with respect to the inflammatory lung lavage parameters LDH and protein did not show a significant difference between MMVF-21 or E-glass and carbon fibers. Analysis of the cell proliferation data was still not done at that time.

After the statistical analysis of the cell proliferation assay has now been performed, it showed that at equal lung burdens of long fibers the carbon fiber exposed rats had induced significantly greater effects on bronchiolar cell proliferation but not on parenchymal (alveolar) cell proliferation. This distinction is important since in rats the alveolar Type 2 cells are the precursor cells for lung tumors, but not the bronchiolar cells. The statistical analysis of these results was performed by comparing those animal groups of the different fiber studies which had the same lung burden in terms of retained fibers longer than 20μm at the end of exposure. These long fibers are the fibers with the greatest carcinogenic potential. Thus, based on the retained fiber lung burden at the end of exposure the following groups were compared: Carbon low *vs.* E-glass low *vs.* MMVF-21 medium; carbon medium *vs.* E-glass medium. Comparisons between others groups cannot be made since the retained lung burdens are too different, as can be seen in the Table 10 below. Since the parenchymal cell labeling index did not show significant differences between the carbon fibers and the other fiber types, only the results of the Tukey test for bronchiolar cell labeling at 15 weeks post-exposure are summarized in Table 11. As is shown there, the E-glass low and carbon low groups are not significantly different from each other, whereas MMVF-21 mid-dose shows significantly lower proliferation than carbon low, but was not different from e-glass low; E-glass medium showed significantly lower bronchiolar cell proliferation than carbon medium. There was one additional significant difference between E-glass medium and carbon medium at 8 weeks post exposure, where E-glass again was significantly lower than carbon.

A surprising finding from comparing the carbon fiber results with the MMVF-21 and E-glass fibers is a significantly lower induction of inflammatory cells (neutrophils) in the lung (analysis of lung lavage cells) by carbon fibers compared to E-glass and MMVF-21 (figure1). This will be addressed below.

With respect to the retention of carbon fibers in the lung there is a dose-dependent increase of the retention halftime for fibers >20 μm (Table 12) from about 250 days for the low dose to about 500 days for the mid-dose and more than 1000 days for the high dose carbon fibers. This needs to be compared with retention halftimes for E-glass of about 60 days for all doses and for MMVF-21 of about 30 days for the low and mid-dose and 140 days for the high dose. Despite these long retention halftimes for carbon fibers, there is little accumulation of these fibers in the lymph nodes at 15 weeks post-exposure (Table 13), ranging between 0.3 and 0.4% for fibers greater than 20 μm long of the 3 dose levels, so there is no dose-dependent increase in that endpoint. For shorter fibers, there is a somewhat greater lymph node accumulation between 1.4 and 2.2% (except at the high dose for fibers less than 5 μm where the value is 7.1%). Unfortunately, no respective data for MMVF-21 and E-glass are available, but a subchronic study with amosite also conducted by the Fraunhofer Institute, showed lymph nodal retention for fibers longer than 20 μm to be lower (about 0.1%), and for all fibers it was also lower (0.4%) at 15 weeks post-exposure.

Thus, the long retention halftime in the lung and the translocation of carbon fibers to the lymph nodes point to a long biopersistence of carbon fibers in the lung accompanied by some translocation of the fibers to the interstitium. It is surprising that despite this long biopersistence and interstitial translocation, no increased fibrotic responses in the lung have been seen. It appears that E-glass, indeed, shows a greater fibrotic score than carbon at 15 weeks post-exposure (Fig. 2), however, this difference is statistically not significant.

The long biopersistence of carbon fibers and the increased cell proliferation of the bronchiolar cells make it difficult to predict what the outcome of a chronic study with respect to carcinogenicity would be. If we were dealing with a vitreous fiber, my prediction for a chronic rat inhalation study based on these findings would be that lung tumors will be induced in rats in a two-year study at the highest concentration. This prediction is mainly based on the long biopersistence of carbon fibers in the lung combined with a high dose effect, and less on bronchiolar cell proliferation, since bronchiolar cell proliferation is not viewed as indicating proliferation of precursor cells for lung tumors in rats.

However, recent developments in our thinking of using outcomes of short-term assays with fibers to predict long-term effects has undergone some changes, as can be judged from extensive ongoing discussions among a group of experts: The International Life Sciences Institute (ILSI) –

with sponsorship of the US-EPA - has convened two meetings and is preparing a document on "Testing of fibrous particles: Short-term assays". I am a member of this Working Group, and our group discussions focus on vitreous and asbestos fibers since this is the area where most experience and data have been accumulated. Organic fibers are viewed differently by this group, and carbon fibers are also viewed as outside this vitreous fiber category, and therefore require more data to conduct a final evaluation regarding carcinogenicity. Significant gaps need to be filled in order to understand better possibly different patho-physiological mechanisms for these types of fibers compared to vitreous fibers. These new developments in fiber toxicology *vis-à-vis* our well-established data set on the correlation between fiber biopersistence and adverse health effects for vitreous fibers have to be appropriately considered here. For this reason, I am hesitant to categorically predict that these carbon fibers are likely to be carcinogenic, which I would do if these results were from a biopersistent vitreous fiber. A key consideration should be that alveolar cell proliferation was not increased compared to vitreous fibers.

I will try to rationalize my response by drawing parallels to what we know about spherical carbon particles. Spherical carbon particles tested in long-term rat inhalation studies have been found to induce lung tumors only at concentrations which resulted in a phenomenon termed "lung particle overload". The same is true for any fibrous particle of low cytotoxicity, *e.g.*, TiO_2, which will induce lung tumors only in rats when an overload situation is achieved, but not in other rodents; for this reason, based on the animal data, TiO_2 specifically has been de-listed as a potential human carcinogen by EPA. Cytotoxicity of the particles is the key to differentiate potential carcinogenic compounds: When a cytotoxic particle such as crystalline silica (SiO_2) was tested in a long-term inhalation study in rats, it induced lung tumors at much lower concentrations than TiO_2 or carbon; and these SiO_2 concentrations clearly did not induce what is defined as "lung particle overload". Based on the animal experimental data and also on available positive epidemiological data from occupational expopsures, SiO_2 is characterized as a known human lung carcinogen.

Typical findings in positive (lung tumor-inducing) rat inhalation studies with particles are always the same, regardless of whether lung overload situations are generated with very high doses of more benign particles such as carbon and TiO_2 or with lower lung doses of a cytotoxic particle such as SiO_2: Chronic pulmonary inflammation, increased alveolar cell proliferation, interstitial fibrosis, lung tumors. In contrast, low lung doses of spherical particles of low cytotoxicity, such as carbon or TiO_2, do not induce these effects.

In the present subchronic carbon fiber inhalation study, the lung burdens of the low dose group (about 300 μg, Table 13) was clearly not in the overload range by mass, whereas the mid-dose (lung burden about 1.2 mg) and certainly the high dose (lung burden greater than 3 mg) were

in the overload range. Despite the high lung burdens in the mid- and high-dose carbon fiber groups, alveolar inflammation at one week post-exposure as judged by the percent of neutrophils in lung lavage, was significantly lower than that of MMVF-21 and of E-glass; this is inconsistent with what one would expect from a carcinogenic fiber. This inconsistency, indeed, may point to the fact that carbon fibers — as discussed presently within the afore mentioned ILSI Working Group — may interact differently with target cells in the lung than glass or asbestos fibers.

On the other hand, the significance of the increased bronchiolar cell proliferation at 15 weeks post-exposure may either indicate a potential for significant adverse effects, such as fibrosis, later on— although the fibrotic score was not significantly different from MMVF-21 — or may point to a "physiological" response in the conducting airways towards the carbon material. In the context of cell proliferation, it should kept in mind that parenchymal cell proliferation and pleural cell proliferation – which are more predictive for tumorigenesis by inhaled fibrous particles in rats - were not significantly increased against MMVF-21.

Overall, the outcome of this subchronic carbon fiber study clearly shows increased biopersistence of the carbon fiber compared to vitreous fibers, and increased bronchiolar cell proliferation at late timepoints post-exposure. The lung inflammatory response and the fibrotic score, in contrast, are less than would be expected from a persistent fibrous particle with fibrotic and carcinogenic potential. Given the present uncertainty among experts of a fiber working group on how to categorize carbon fibers based solely on the outcome of a short-term assay, a prediction regarding a carcinogenic potential can only be made with great caution, lacking data of a chronic carbon fiber study. Based on our present knowledge, it is my view that inhaled concentrations of carbon fibers which do not cause lung overload will not induce lung tumors in a chronic rat inhalation study. This means that carbon fibers seem to induce pulmonary effects similar to those of low cytotoxic particles. Accepting this concept and applying an adequate safety factor for human exposures to carbon fibers, workplace conditions can be created through appropriate surveillance and engineering conditions which do not exceed "safe" airborne concentrations.

Table 10. Retained lung burdens of different fibers following subchronic inhalation (13 weeks) in rats at different timepoints post-exposure. (Fiber numbers/lung x 10^6)

	1 week post-exposure			8 weeks post-exposure			15 weeks post-exposure		
	Carbon	MMVF-21	E-glass	Carbon	MMVF-21	E-glass	Carbon	MMVF-21	E-glass
Low	3.3	0.9	2.9	3.1	0.35	1.7	2.5	0.1	1.0
Mid	10.8	3.9	10.1	10.4	1.9	5.4	9.5	0.45	3.8
High	31.5	5.7	17.0	28.3	5.5	13.3	29.4	3.6	6.5

Table 11. Significant differences between carbon fiber, E-glass and MMVF-21 for bronchiolar cell proliferation at 15 weeks post-exposure

	E-glass Low	E-glass Mid	MMVF Mid
Carbon Low	Not significant	Not applicable *(different lung burden)*	Carbon significantly higher
Carbon Mid	Not applicable *(different lung burden)*	Carbon significantly higher	Not applicable *(different lung burden)*

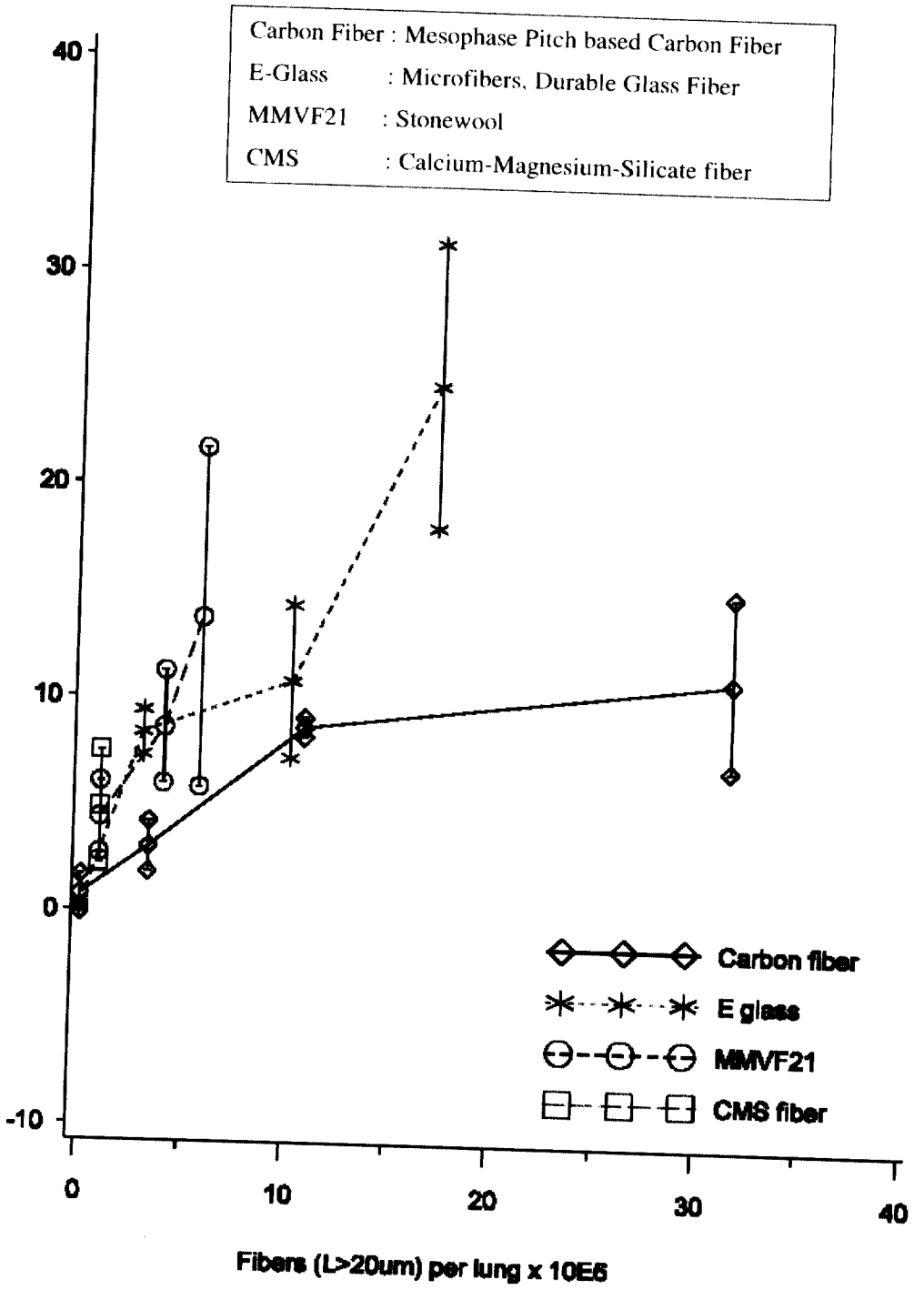

Fig.3 **% PMNs at 1 Week postexposure**

Table 12. Clearance half-time and 95% confidence limit (95%C.L.) of the elimination of test fibers (calculation from lung retention data listed in Table 13)

Group	Half-time in days calculated from			
	Number of fibres Mean (95%C.L.)	Number of WHO fibres Mean (95%C.L.)	Number of fibres (L>20µm) Mean (95%C.L.)	Number of particles Mean (95%C.L.)
Linear regression of log-values				
Carbon fiber Low	88 (69 - 122)	113 (86 - 168)	261 (158 - 765)	86 (46 - 684)
Carbon fiber Med	76 (65 - 90)	97 (84 - 114)	639 (244 - ∞)	57 (44 - 82)
Carbon fiber High	113 (93 - 144)	176 (84 - 114)	>1000 (228 - ∞)	83 (63 - 119)
Nonlinear regression (exponential fit) according to EU protocol ECB/TM/26				
Carbon fiber Low	83 (65 - 100) [1.00]	103 (79 - 127) [1.00]	248 (100 - 397) [0.99]	83 (56 - 110) [0.98]
Carbon fiber Med	75 (61 - 88) [0.99]	99 (82 - 116) [0.99]	478 (0 - 966) [1.00]	57 (45 - 70) [0.96]
Carbon fiber High	134 (95 - 173) [1.00]	264 (128 - 401) [1.00]	>1000 (0 - ∞) [0.99]	83 (58 - 107) [0.97]

[0 xx] R-Square (R-square x 100 is the percentage of the variance which can be explained by the single exponential regression model)

Table 13. Analysis of fibers in the lungs and lung-associated lymph nodes (LALN)

Group	Sacrifice after end of exposure [weeks]		Number of Fibers [10^6/lung]					Particles [10^6/lung]	Estimated mass from SEM analysis [µg/lung]*	
			All	L≤5 µm	L=5-20 µm	WHO	L> 20µm		Fibers	Particles
Control	1	Mean	0.0	0.0	0.0	0.0	0.000	0.0	0	0
		SD	0.0	0.0	0.0	0.0	0.001	0.0	0	0
	8	Mean	0.0	0.0	0.0	0.0	0.000	0.0	0	0
		SD	0.0	0.0	0.0	0.0	0.000	0.0	0	0
	15	Mean	0.0	0.0	0.0	0.0	0.000	0.0	0	0
		SD	0.0	0.0	0.0	0.0	0.000	0.0	0	0
	15 LALN	Mean	0.00	0.00	0.00	0.00	0.000	0.00	0.0	0.0
Carbon fiber low	1	Mean	17.9	4.8	9.9	13.1	3.267	5.6	267	28
		SD	3.6	1.8	1.7	2.0	0.452	1.5	27	8
	8	Mean	13.6	2.0	8.5	11.6	3.079	7.1	210	22
		SD	2.7	1.0	2.0	1.7	0.393	8.2	19	16
	15	Mean	8.2	1.0	4.7	7.2	2.512	2.5	149	9
		SD	0.2	0.2	0.3	0.3	0.267	0.2	15	1
	15 LALN	Mean	0.07	0.01	0.06	0.07	0.008	0.00	0.8	0.0
Carbon fiber med	1	Mean	87.6	25.6	51.2	62.0	10.758	31.9	1069	154
		SD	7.3	7.0	4.7	5.2	2.027	6.6	105	40
	8	Mean	48.9	8.6	29.9	40.3	10.411	17.1	687	67
		SD	3.9	1.8	1.4	2.5	1.063	7.1	47	21
	15	Mean	35.7	5.1	21.1	30.7	9.540	9.8	641	56
		SD	3.3	1.6	2.0	2.1	0.480	1.8	89	16
	15 LALN	Mean	0.65	0.19	0.43	0.46	0.035	0.47	4.7	7.4
Carbon fiber high	1	Mean	205.1	59.9	113.7	145.2	31.498	71.1	2815	351
		SD	26.3	19.4	15.0	23.3	11.415	12.5	495	71
	8	Mean	137.4	30.0	79.1	107.4	28.264	45.6	1929	166
		SD	5.8	3.9	4.5	5.9	2.471	8.6	235	34
	15	Mean	111.8	13.9	68.4	97.9	29.428	31.3	2150	182
		SD	7.0	3.9	3.6	4.3	1.907	6.1	296	99
	15 LALN	Mean	2.17	1.08	1.02	1.09	0.070	0.91	9.4	6.7

SD Standard deviation

* Definition of fibers and particles and method for estimation of mass see **3.4.3**

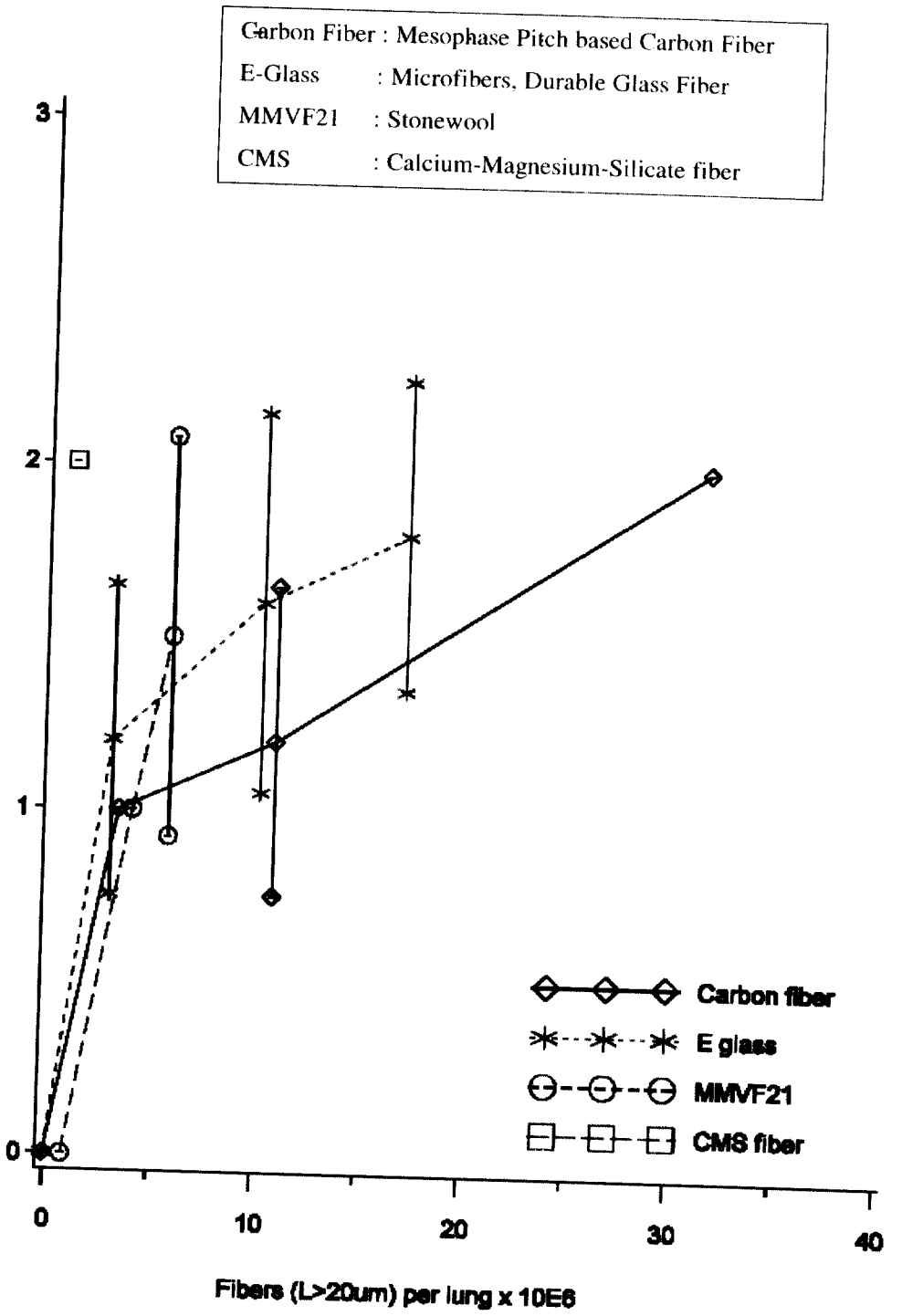

Fig.4 **EPS grade at 15 Weeks postexposure**

Original 1 of 2

Fraunhofer Institut
Toxikologie und
Experimentelle Medizin

Final Report

on

Subchronic Inhalation Toxicity of Carbon Fibers in Rats

Fraunhofer ITEM Study Number: 02G02022

Sponsor

[redacted]

Test Facility

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September 2003

This report consists of 153 pages

CONTENTS PAGE

Tables

Figures

SUMMARY AND CONCLUSIONS

General

The aim of the study was to investigate the intrinsic toxicity of carbon fibers with a mean diameter of about 1μm in rats after subchronic inhalation according to the protocol of the European Chemicals Bureau (ECB/TM 16 (97) rev. 1).

Rats were exposed 6 hours per day, 5 days per week for 3 months. The target dose was an exposure to 15, 50 and 150 fibers/ml at a fiber length > 20 µm. **Table 3** shows that these concentrations were reached. After exposure of rats to the test fiber no biological effects were observed except those in the lungs.

In the medium and high dose groups a statistically significant increase of the lung weight was observed up to 3 months postexposure (see **Table 6**). In the low dose group a significant increase was seen only at the first sacrifice date 1 week postexposure.

After 3 months of exposure the lung retention of WHO fibers in fibers per lung was about 13 x 10^6, 62 x 10^6 and 145 x 10^6 for low, medium, and high dose, respectively (see **Table 7**). The corresponding values for long fibers (length > 20 µm) were about 3.3 x 10^6, 10.8 x 10^6 and 31.5 x 10^6 for the three groups. The corresponding values for the retained mass of fibers (estimation from the number and size of fibers in the lung ash) in the lungs in mg/lung were 0.27, 1.1, and 2.8 for low, medium, and high dose, respectively. The estimated particle mass was less than 20% of the corresponding fiber mass in the lungs for all fiber groups.

After 3 months of recovery the WHO fiber concentration in the lungs decreased to 55%, 50% and 67% for the low, medium and high dose group, respectively. For the long fiber fraction with length > 20μm the corresponding data were 77%, 89% and 93% for the three dose groups. The elimination of fibers is slower in the high dose group compared to the low and medium dose group. Additionally the elimination of the long fiber fraction is much slower than for the WHO fiber fraction, which is different to the clearance behavior of man-made vitreous fibers. For most MMVFs a faster clearance was detected for the long fiber fraction compared to the WHO fiber fraction.

The effects on biochemical parameters (LDH and protein) in the bronchoalveolar lavage fluid (BALF) were statistically significant at the end of the 3 months exposure and at 8 and 15 weeks post-exposure for the high dose groups (**Table 11**). For the medium dose group the effect on protein was statistically significant and the effect on LDH was not significant for all sacrifice dates. For the low dose group only the protein was increased significantly only once at end of exposure. The effect on ß-glucuronidase was significant only for the high dose group at end of 3 months exposure but not for later sacrifice dates. For the low and medium dose group the small increase of ß-glucuronidase was not significant for all sacrifice dates.

For medium and high dose fiber groups a statistically significant increase of neutrophil PMN and a

reduction of the percentage of macrophages in the BALF was observed for all sacrifice dates, but PMN levels were lower for 8 weeks and 15 weeks postexposure (**Table 12**). There was also a significant increase in lymphocytes in the mid and high dose groups up to 3 months postexposure. No significant effect was seen in the low dose group.

Histopathology

At the termination of the 3-month exposure period, very slight to moderate morphological changes were diagnosed in the lungs (**Table 15**). These changes included alveolar macrophage aggregation and/or microgranulomas at the bronchiolo-alveolar junction. At the end of exposure evidence of early interstitial fibrosis was detected for 2 rats of the medium dose group and for all 5 rats of the high dose group (**Table 15**). At 8 and 15 weeks postexposure this effect decreased to one rat of the medium dose group, but was retained at 5 rats for the high dose group.

The cell proliferation was enhanced at end of exposure in the high dose group for the bronchiolar epithelium, for alveolar parenchymal cells and for pleural cells, in the medium group for the bronchiolar epithelium and for alveolar parenchymal cells, and in the low dose group only for the bronchiolar epithelium (**Table 16**).

For the alveolar parenchymal cells the increase of cell proliferation persisted at significant levels for the high dose group up to 3 months, but for the medium dose group it was no longer significant at 3 months postexposure. For the cell proliferation of the bronchiolar epithelium the increase retained significant up to 3 months postexposure for all treatment groups. This effect corresponds to the histopathological finding of bronchio-alveolar hyperplasia, which is a cellular response to fiber deposition and retention at the bronchioalveolar epithelium. This is an protective effect to increase the mucus production for enhancement of clearance of fibers in that region.

Overall assessment

In a previous study at Fraunhofer ITEM (Bellmann et al, 2003) three other fiber types (E-glass microfibers, stonewool MMVF21 and the biosoluble Calcium-Magnesium-Silicate [CMS] fiber) were investigated in a 90 day inhalation study using the same protocol (ECB/TM 16(97) rev. 1).

The effects for different parameters are plotted versus the retained number of long fibers (length>20μm) in Figures in Appendix 10.

The results of comparison the effects of carbon fibers to that of E-glass, MMVF21 and CMS are summarized in **Table 17** (see page 61).

CERTIFICATION

Statement of Principal Scientists

Study No.: 02G02022
Test Item: Carbon fibers (lot P-173)
Title of the Report: Subchronic Inhalation Toxicity of Carbon Fibers in Rats

We, the undersigned, hereby declare that the work in this study was performed by us or under our supervision according to the procedures herein described and that this report provides a correct and faithful record of the results obtained.

Fraunhofer Institute of Toxicology and Experimental Medicine		Signature/Date
Aerosol Scientist:	Dr. rer. nat. G. Pohlmann	29.5.3
Laboratory Animal Veterinarian:	Prof. Dr. med. vet. Dasenbrock	26.9.03
Biochemist:	Dr. rer. nat. O. Creutzenberg	26/9/03
Clinical Chemist:	Dr. med. vet. T. Hansen	26.09.03
Pathologist:	Dr. med. vet. H. Ernst	29.09.03

Statement of Study Director

Study No.: 02G02022
Test Item: Carbon fibers (lot P-173)
Title of the Report: Subchronic Inhalation Toxicity of Carbon Fibers in Rats

The study described in this report was conducted in compliance with Principles of Good Laboratory Practice (German Chemicals Law § 19a Appendix 1 pp. 2119-2129, June 28, 2002).
The study followed the regulations of the German Animal Protection Law (Tierschutzgesetz of May 25, 1998).
I, the undersigned, hereby declare that this report provides a correct and faithful record of the results obtained. I accept the responsibility for the validity of the study.

Date

30.09.2003

Signature

Fraunhofer Institute
Toxicology and Experimental Medicine (Fraunhofer ITEM)
Study Director:
Dr. B. Bellmann

Quality Assurance Statement

Study No.: 02G02022
Test Item: Carbon fibers (lot P-173)
Title of the Report: Subchronic Inhalation Toxicity of Carbon Fibers in Rats

The Quality Assurance Unit (QAU) hereby declares that the study was inspected at intervals adequate to ensure the integrity of the study and the findings reported to the Study Director and the management of Fraunhofer ITEM. The exact dates of inspections and report are given below. The report describes the methods and procedures used in the study and the documented results accurately reflect the raw data of the study.

Date of Inspection	Type of Inspection	Date of Report to Study Director/ Management
05.06.-14.08.2002	Study Plan	14.08.2002
22.08.2002	Exposure, Raw data	22.08.2002
12.09.2002	SEM Sample Preparation	13.09.2002
16.10.2002	Exposure, Fiber counting	17.10.2002
18.-22.10.2002	SEM fibre measurement	22.10.2002
06.11.2002	Exposure, Raw data	11.11.2002
15.11.2002	BrdU Application	15.11.2002
18.-19.11.2002	Necropsy 3 Months, BAL	19.11.2002
28.10-28.11.2002	Raw data bodyweight	29.11.2002
29.11.2002	SEM fibre measurement	29.11.2002
06.-08.01.2003	Necropsy, BAL, BrdU, Clin. Chemistry	09.01.2003
10.-12.02.2003	Draft Report Stage 1	12.02.2003
13.-17.02.2003	Final Report Stage 1	17.02.2003
21.-27.02.2003	Necropsy, BAL, BrdU, Clin. Chemistry	28.02.2003
27.08.2003	SEM, Sample preparation	28.08.2003
11.-16.09.2003	Fiber analysis SEM	17.09.2003
24.-26.09.2003	Final report	26.09.2003

Date

30/9/03

Signature

p.p.

Fraunhofer Institute
Toxicology and Experimental Medicine (Fraunhofer ITEM)
Head, Quality Assurance Unit
Dr. M. Ketkar

Copy of the GLP CERTIFICATE

Niedersächsisches
Landesamt für Ökologie

Gute Laborpraxis/Good Laboratory Practice

GLP-Bescheinigung/Statement of GLP Compliance

(gemäß/according to § 19 b Abs.1 Chemikaliengesetz)

Eine GLP-Inspektion zur Überwachung der Einhaltung der GLP-Grundsätze gemäß Chemikaliengesetz bzw. Richtlinie 88/320/EG wurde durchgeführt in:

Assessment of conformity with GLP according to Chemikaliengesetz and Directive 88/320/EEC at:

☒ Prüfeinrichtung / Test facility ☐ Prüfstandort / Test site

Fraunhofer-Institut für Toxikologie und Experimentelle Medizin
in D-30625 Hannover, Nikolai-Fuchs-Straße 1

(Unverwechselbare Bezeichnung und Adresse/Unequivocal name and address)

Prüfungen nach Kategorien/Areas of Expertise (gemäß/according ChemVwV-GLP Nr. 5.3/OECD guidance)

1 - Prüfungen zur Bestimmung der physikalisch-chemischen Eigenschaften und Gehaltsbestimmungen
2 - Prüfungen zur Bestimmung der toxikologischen Eigenschaften
3 - Prüfungen zur Bestimmung der erbgutverändernden Eigenschaften (in vitro und in vivo)
5 - Prüfungen zum Verhalten im Boden, im Wasser und in der Luft, Prüfungen zur Bioakkumulation und zur Metabolisierung
8 - Analytische Prüfungen an biologischen Materialien
9 - Sonstige Prüfungen:
  - Sicherheitspharmakologie, Teilbereich Lunge
  - Molekulartoxikologische und molekularbiologische Prüfungen zu Chemikalien, Pflanzenschutzmittel-Wirkstoffen, Bioziden und pharmakologischen Wirkstoffen

Datum der Inspektion / Date of Inspection
(Tag.Monat.Jahr / day.month.year)

25. – 27. November 2002

Die/Der genannte Prüfeinrichtung/Prüfstandort befindet sich im nationalen GLP-Überwachungsverfahren und wird regelmäßig auf Einhaltung der GLP-Grundsätze überwacht.

The above mentioned test facility / test site is included in the national GLP Compliance Programme and is inspected on a regular basis.

Auf der Grundlage des Inspektionsberichtes wird hiermit bestätigt, dass in dieser Prüfeinrichtung/diesem Prüfstandort die oben genannten Prüfungen unter Einhaltung der GLP-Grundsätze durchgeführt werden können.

Based on the inspection report it can be confirmed, that this test facility / test site is able to conduct the aforementioned studies in compliance with the Principles of GLP.

Niedersächsisches Landesamt für Ökologie/Lower Saxony State Agency for Ecology
Hildesheim, 06.02.03
Im Auftrage

Teuteberg
(Bauamtmann)

NIEDERSÄCHSISCHES LANDESAMT FÜR ÖKOLOGIE - KANZLEI - 4

1 PREFACE

1.1 General Information

Fraunhofer ITEM Study No:	02G02022
Test Facility:	Animal Room No. T1.030a Room T1.108/109 (Laboratory) Room T1.07 (Necropsy area)
Test Item:	Carbon fibers (lot P-173)
Fraunhofer ITEM Study Director:	Dr. rer. nat. B. Bellmann
Sponsor's Project Monitor:	Prof. Dr. G. Oberdörster
Study Initiation Date:	August 14, 2002
Experimental Starting Date (Start of animal exposure):	August 15, 2002
Experimental Completion Date Last date on which data are collected directly from the study:	September 29, 2003
Study Completion Date:	September 30, 2003

1.2 Guidelines for Conduct of Study

This animal study was conducted in compliance with the Principles of Good Laboratory Practice (OECD January 21, 1998 and German Chemicals Law § 19a Appendix 1 pp. 2119-2129, June 28, 2002).

The study followed the regulations of the German Animal Protection Law (Tierschutzgesetz of May 25, 1998).

1.3 Study Staff

Study Director:	Dr. rer. nat. B. Bellmann
Deputy Study Director:	Dr. rer. nat. O. Creutzenberg
Aerosol Scientist:	Dr. rer. nat. G. Pohlmann
Laboratory Animal Veterinarians:	Prof. Dr. med. vet. C. Dasenbrock
	Dr. med. vet. Th. Tillmann
Biochemist:	Dr. rer. nat. O. Creutzenberg
Clinical Chemist:	Prof. Dr. rer. nat. W. Bartsch
	Dr. med. vet. T. Hansen
Pathologists:	Dr. med. vet. H. Ernst
	Dr. med. vet. S. Rittinghausen
Quality Assurance:	Dr. M.B. Ketkar

2 GENERAL STUDY INFORMATION

2.1 Objective

The objective of this non-clinical health and environmental safety study was to investigate the intrinsic toxicity of carbon fibers with a mean diameter of about 1 μm.

Strategies

The biological activity (inflammatory, cell proliferative and fibrotic potential) of carbon fibers was investigated after subchronic inhalative exposure in rats.

The investigation followed in principle the preliminary guideline of the European Chemicals Bureau (ECB/TM 16 (97) rev. 1) for the investigation of the toxicity of Synthetic Mineral Fibers (SMF) in rats after subchronic inhalation. The results can be compared to previous studies conducted at Fraunhofer ITEM with different SMF and with amosite asbestos using that protocol (Fraunhofer ITA, 2001, Bellmann et al., 2001, Brown et al. 2001, Bellmann et al., 2003).

2.2 Overall Design

A subchronic inhalation study in rats was performed for three dose groups of the carbon fiber sample. The 3 months exposure period was followed by a 3 months postexposure observation period.

The rats were exposed 6 hours per day, 5 days per week for 3 months to a fiber aerosol concentration of 15, 50 and 150 fibers/ml (fiber length > 20μm). Within a 3 month observation period after end of 3 months exposure the rats were sacrificed at different intervals (see **Table 2**).

2.3 Primary Parameters

The endpoints at different post exposure dates included:

Body weight, lung weight

Bronchoalveolar lavage

- cell concentration (macrophages, neutrophils, eosinophils and lymphocytes)
- biochemical parameters (lactic dehydrogenase (LDH), ß-glucuronidase, and total protein)

Conventional histopathology of lung and cell proliferation measurement test (BrdU S-phase response assay).

Lung burden was analyzed in terms of the number and bivariate size distribution of fibers and particles in the lungs at end of exposure and at different time intervals after exposure. The half-time ($T_{1/2}$) for the elimination of fibers of different length fractions was calculated using fiber burden at 1, 8, and 15 weeks postexposure.

2.4 Method Guideline

The preliminary guideline of the European Commission ECB/TM 16(97) rev. 1 was used with the following modification:

A modified procedure for monitoring the aerosol concentration was used (see **5.2.1**).

3 TEST ITEM

The test item (carbon fibers, lot number P-173) was supplied by the sponsor along with all the necessary information. For the subchronic inhalation study about 200 g of sized test fibers (see **3.2**) were necessary. According to the preliminary EC guideline ECB/TM/16 for SMF the geometric mean diameter (GMD) of the fibers longer than 20 μm in the sized material should be as close to 0.8 μm as possible, for fibers with a density of about 2.4, if technically feasible. However since carbon fibers have a lower density (about 1.5) the GMD could be close to about 1.0 μm, if technically feasible. The sponsor is responsible for the correct identity of the test fibers.

The remaining test item was returned to the sponsor after end of exposure except a sample for storage in the archives of Fraunhofer ITEM.

3.1 Safety Protection, Storage and Handling

Routine hygienic procedures were followed to assure that there were no adverse effects on the health and safety of personnel handling the test fiber.

3.2 Sizing of the Bulk Material

The bulk commercial material obtained from the sponsor was shortened by milling in a Retch® mill. The milled material was used directly for aerosol generation. It was not necessary to use an aerosol separation technique for additional sizing. Previous experience suggested that a considerable fraction might be particles which do not fulfill the fiber definition. The amount of this fraction is dependent on the properties of the test fibers. The goal was to keep the amount of non-fibrous particles less or equal to 20% of mass.

The fraction of respirable fibers was characterized by analyzing the length and diameter of about 400 fibers.

3.3 Characterization of Test Fiber

The test fiber was characterized by a Scanning Electron Microscope (SEM, LEO-430, LEO Elektronenmikroskopie GmbH, Oberkochen, Germany).

A small fraction of the test fibers (about 0.1 mg) was suspended in 10 - 20 ml double-distilled water (containing 0.05% Tween 80). Then the suspension was sonicated (Sonorex RK 510H at 35 kHz and 225 W, Bandelin, Berlin, Germany) for about 30 sec and filtered onto a Nuclepore® filter (25 or 47 mm diameter, pore size 0.2 or 0.4 μm). The preparation of the SEM sample and the counting and sizing is described in section **3.4**.

3.4 SEM Analysis of Fiber Samples

The following procedure was used for stock fibers and fibers in aerosol samples and lung samples.

3.4.1 Preparation of SEM Samples

A part of the filter containing the fiber sample was mounted on an aluminum stub and sputtered (Balzers SCD 030, Balzers Hochvakuumtechnik, Wiesbaden, Germany) with about 30 nm of gold.

3.4.2 Counting and Sizing Rules (for aerosol and lung fibers)

The general guidelines provided by the WHO/EURO (World Health Organisation, Reference Methods For Measuring Airborne Man Made Mineral Fibers (MMMF), prepared by the WHO Regional Office for Europe, Copenhagen (1985)) was followed with the following additional procedures for synthetic mineral fibers.

3.4.3 Length and Diameter

Sizing of length and diameters were performed using a SEM at a magnification of at least 2000. All objects, which were seen at this magnification, were counted. Fibers crossing the boundary of the field of view were counted as follows. Fibers with only one end in the field were weighted as half of a fiber and fibers with neither of their ends in the field were not measured. Diameters of fibers which were seen at 2000 magnification were measured at full screen magnification (usually up to a magnification of 12,000). No lower or upper limit is to be imposed on either length or diameter. The length and diameter were recorded individually for each fiber measured so that the bivariate distribution could be determined. When sizing, an object was accepted as a fiber if the ratio of length to diameter was at least 3:1. All other objects were considered to be particles. There was no truncation in the measurements.

For aerosol and lung samples from length and diameter of fibers the mass was estimated assuming cylindrical geometry of fibers. A density of 1.0 g/cm^3 was used which resulted in the best correspondence between gravimetric aerosol concentration and SEM estimated aerosol concentration.

The results of the size distribution of the aerosol and lung samples are presented as percentile values and as arithmetic and geometric means and standard deviations. The aerodynamic diameter of fibers was calculated from length L, diameter D and density S (using A=2 x LN (L/D)) (Harris and Fraser, 1976) by:

$$Aer.D. = 1.5 * D * \sqrt{\frac{S}{\frac{0.385}{A-0.5} + \frac{1.230}{A+0.5}}}$$

3.4.4 Stopping Rules

Enough fields of view were counted for evaluation so that at least a total of 0.15 mm^2 of the filter surface (for 25 mm diameter) was examined. The following conditions were fulfilled:

1. Fibers: A size selected analysis using a minimum of 100 fibers per category for the 2 length categories ≤ 5 μm; and > 20 μm and a minimum of 200 fibers for the length category 5 - 20 μm was used. The distance between two fields of view for analysis was at least 10 fields. Sizing was stopped when 1 mm^2 of the filter surface was examined, even if the minimum number of fibers was not reached for a category. The raw data file contains the different values for the areas which were analyzed for the 3 length categories. The total number of fibers per filter was determined by

normalizing the surface area counted to the total surface area of the filter.

2. Particles: The recording of particles was stopped when a total of 100 particles were counted.

4 TEST SYSTEM

4.1 Animal Model

Male Wistar rats (strain Crl:WU) were purchased from Charles River Deutschland, Sulzfeld, Germany. The age of the animals at the start of exposure was about 8 - 9 weeks and the weight approximately 200-300g.

Rats were exposed to the test fiber by nose-only inhalation. For a period of 3 weeks prior to exposure animals were trained to become accustomed to nose-only tubes.

Wistar rats were recommended by an EPA workshop (Vu et al., 1996) for use in subchronic and chronic inhalation toxicity studies of fibers. According to protocol ECB/TM/16(97) rev. 1 the use of male Wistar rats is preferred.

4.2 Disease Screening

The animals were received as SPF rats (specific pathogen free). They were maintained under barrier conditions throughout the study, and a subgroup (10 rats) was included for Disease Surveillance. This Disease Surveillance included screening for the presence of antibodies to the following pathogens: Pneumonia virus of mice (PVM), Sialodacryoadenitis (SDA)/ Rat coronavirus (RCV), Mycoplasma pulmonis, Sendai virus (Parainfluenza-1 virus), Kilham rat virus (KRV),Reovirus type 3 (Reo3). The Disease Surveillance was performed at the start of the study, near week 13 and near the end of the study.

4.3 Acclimatization

The animals were allowed to adjust and become acclimatized to the Fraunhofer ITEM environment for approximately four weeks before starting exposure. Upon arrival, all animals were thoroughly examined including the skin and coat, eyes, ears, nose and body openings.

For acceptance of animals into the study the physical condition of the animals were checked by daily clinical observations in addition to the Disease Surveillance (see **4.2**). During this time animals were trained to become accustomed to nose-only tubes.

4.4 Randomization

The animals were allocated to groups on a body weight basis. Prior to the initiation of the treatment the animals were weighed, randomized and grouped by the PROVANTIS software (management of toxicology laboratory data, Instem Life Science System Ltd., Walton Industrial Estate, Stone, Staffs, ST 15 OLT, Great Britain, version 5.0.1). This process assured that the mean group body weights of rats within groups (see **Table 1**) were very similar, at that time.

4.5 Identification

To each animal in the study a unique four or five digit identification number was assigned. These four or five digits are necessary for data storage in the computer system. The number assigned was G0NN where G denotes the one or two digits (1-32) group number, 0 is a common separator and NN is the two digits (01-99) consecutive animal number. All data collected from an animal were filed under that number. Each animal was permanently identified by a metal plate containing the study number, the group number and the consecutive animal number on the cage corresponding to that identification number. The right ear of the animal was tattooed with the group number and the left ear with a two digit animal number.

4.6 Housing and Maintenance

Animals were housed in Makrolon® (polycarbonate) cages type III (37.5 x 21.5 x 20 cm, two rats per cage) and were maintained under barrier conditions. Cages and absorbing softwood bedding material (Altromin 3/4, Altromin International, Lage, Germany) were changed twice a week or more often when necessary. Drinking water from the Hannover city water supplier was offered ad libitum fresh weekly or more often when necessary in a Makrolon® bottle fitted with a stainless steel nipple top with a hole approximately 0.5 mm in diameter. As diet a commercial chow in pellet form was used, identified as "Altromin 1324 N spec. prepared", purchased from Altromin International, Lage, Germany. Diet was offered ad libitum fresh weekly or more often when necessary.

The temperature and the relative humidity of the animal room was monitored electronically and recorded on a continuous basis. The limits were set at 22 ± 2°C for temperature and 55% ± 15% for relative humidity. A 12-hour light/dark cycle was used for lighting controlled by an automatic timing device.

4.7 Disposal

Biological waste material including food, bedding, and other disposable materials generated in the animal facility were collected in a special container and disposed of in compliance with local, state, and federal regulations.

4.8 Daily and Weekly Observations

All animals were observed in their cages daily. Animals were also removed from the cages once a week (during exposure period twice weekly), and carefully examined for abnormalities.

4.9 Body Weight Data

Individual body weights were recorded to the nearest 0.1 g once a week up to 3 months and thereafter every 2 weeks throughout the study for all animals. All body weight data were collected using electronic balances, interfaced with a computer and programmed for direct on line data acquisition using the Provantis software (see **4.4**).

4.10 Data Collection and Documentation

Body weights, lung wet weights, weights of the lower half trachea, the BAL data and the histopathological findings were recorded as computer output and/or in bound laboratory notebooks. The reports of body weights, lung weights, LDH, ß-Glucuronidase, and total protein were produced by the Provantis software. The reports of histopathological investigations were obtained from PLACES software.

5 STUDY DESIGN

5.1 General Outline

The study design is shown in **Table 1**.

Table 1 Study design of the subchronic inhalation study, followed by a 3 months observation period

Exposure group	Group No.	Fiber (Length>20μm) concentration (F/ml)	Gravimetric concentration* (mg/m^3)	Number of animals
Control (filtered air)	1	-	-	32
Carbon fibers low	2	~ 15	~ 2.5	32
Carbon fibers medium	3	~ 50	~ 7.5	32
Carbon fibers high	4	~ 150	~ 25	32
Disease Surveillance				10
Total				138

* Estimation based on SEM analysis of pretest for aerosol generation

5.2 Aerosol Generation and Exposure

The test fiber was given to the rats by "nose-only - inhalation". For each nose-only exposure unit the fiber aerosol was generated by a high-pressure pneumatic disperser. The disperser was fed with the test fiber under computerized control, i.e. with feedback to the actual aerosol concentrations measured by an aerosol photometer (see **Fig. 1**). The photometer gave a scatter light signal which is nearly proportional to the particle concentration, if the particle size distribution is constant. The ratio between photometer signal and concentration was determined throughout the study by comparing to gravimetric and numerical fiber concentration.

The aerosol of the test fiber was neutralized by a Ni-63 source to reduce the charge on the fibers. The aerosol was given to the rats by a flow-past nose/snout-only inhalation exposure system which was used for previous fiber inhalation studies at Fraunhofer ITEM. In this system, the fiber aerosol is supplied to each animal individually, and exhaled air is immediately exhausted. The airflow to each animal was approximately 1 l/min which is calculated to be laminar. Therefore according to the protocol ECB/TM/16(97) rev. 1 it was not necessary to measure the oxygen concentration. The airflow, the temperature and the humidity were monitored continuously and were stored as 20 min. mean values.

The animals were restrained in Battelle type polycarbonate tubes; with this system animals in the supply tubes keep their noses close to airflow at the opening of the tubes. The exposure of animals was performed in identical exposure chambers of cylindrical shape, each housing up to 48 animals (3 levels for 16 animals each). Control animals were exposed in identical units to filtered air only.

The duration of exposure was 6 hr/day, 5 days/week for 3 months to three fiber aerosol

concentrations of about 15, 50 or 150 fibers/ml (fiber length > 20 μm). There was no exposure on October 3, 2002 which is a public holiday in Germany. The mean value of the fiber concentration was within +/- 10% of the target value (see **9.1**). Before starting the exposure of rats the aerosol concentration (gravimetric and number of fibers and particles) was tested to get an appropriate fiber concentration and to obtain information about the concentration of particles from begin of the study.

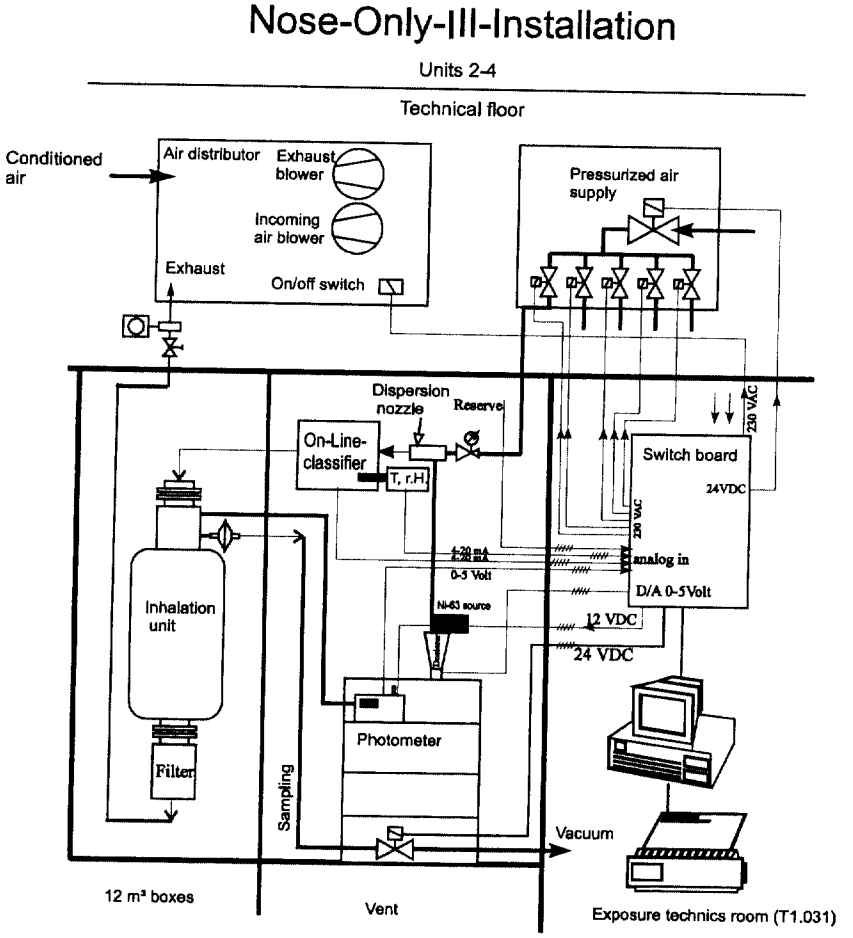

Fig. 1 Experimental set-up of nose-only inhalation system

5.2.1 Monitoring Aerosol Concentration and Fiber Dimensions

The aerosol was monitored (A) by an aerosol photometer (online), (B) by determination of numerical concentration (SEM analysis of filter samples) and (C) by determination of gravimetric concentration (weighing of filter samples).

The number of filter samples for gravimetric analysis (glass fiber filter, Sartorius) and for SEM analysis (Nuclepore filter, pore size 0.8 μm) was used according to the protocol ECB/TM16(97) rev. 1.

Two gravimetric samples per dose group and per exposure day were used.

For one filter the sampling time was about two hours and for the other filter sampling time was timely in coincidence with the sample for fiber counting. For fiber counting and sizing the filter sampling time

is dependent on the fiber concentration (20 to 60 minutes for the dose groups of the test fiber in this study). For the low dose group the filter load of the 60 min filter sample was not sufficient for a precise weighing, therefore only the about 2 hour sampling time was used timely in coincidence with the sample for fiber counting.

For SEM analysis one filter per exposure day and per exposure group was sampled. In the first week all five filters per exposure group, in the following weeks 2 filters were analyzed. The fiber load on the aerosol filter may not be equally distributed. For this reason fibers were resuspended in water (containing 0.05% Tween® 80) and filtered on another Nuclepore® filter.

For the five filter samples in the first week and for two filter samples per week in the next weeks only the concentration of long fibers (L > 20 μm) was analyzed by counting of at least 100 long fibers.

This procedure is a modification of the EU protocol, which was also used in a calibration study for the EU at Fraunhofer ITEM (2001). Additionally, for one of these filter samples per group and week the fiber analysis was performed by counting and sizing including non-fibrous particles. The fiber concentration and the size distribution of fibers and particles were analyzed in SEM as described in the Section **3.4**.

5.3 Study Duration

The subchronic inhalation was performed from August 15 to November 14, 2002. The last sacrifice was on February 26, 2003.

5.4 Endpoints

The endpoints and the number of animals assigned for these investigations are listed in Table 2.

The study was subdivided into the following investigations:

1. Lung burden of test fibers in right lung lobes at end of exposure, 5 animals; in addition 10 animals were reserved for optional analysis at 2 different post-exposure sacrifice dates.
2. Bronchoalveolar lavage at 3 different sacrifice dates with a total of 15 animals per group.
3. Conventional lung histopathology and a cell proliferation measurement of the lung terminal bronchial airways, pleural cells and parenchymal cells by BrdU (S - phase response) using the left lung lobe of the same animals which were scheduled for lung burden analysis.
4. Gross necropsy findings and organ weights of all sacrificed animals.
5. Two animals were used as reserve animals in the event that animals died during the study.

For the cell proliferation measurement minipumps for administration of BrdU were implanted one day after end of exposure 4 days prior sacrifice. Therefore the first sacrifice date for histopathology and lung burden analysis was 5 days postexposure.

Table 2 List of investigations in the subchronic inhalation study
Exposure was terminated after 3 months; subgroups were kept for further 3 months for post-exposure investigations

Investigation	Test required after start of exposure [months]		Group	Number of animals per group for single investigation	Consecutive number of animals per group scheduled for investigation	
	Stage 1	Stage 2			Stage 1	Stage 2
Lung burden of test fibers	3	4.5; 6	1-4	5 [a]	1-5	6-10; 11-15
Bronchoalveolar lavage	3	4.5; 6	1-4	5	16-20	21-25; 26-30
Histopathology/ BrdU	3	4.5; 6	1-4	5	1-5	6-10; 11-15
Reserve animals			1-4	2	31,32	

[a] The left lung lobe of animals scheduled for fiber burden analysis was used for histopathology.

6 METHODS

6.1 Gross Pathology/Necropsy

All animals were subjected to a complete necropsy, which included careful examination of the external surface of the body, all orifices, and the cranial, thoracic and abdominal cavities and their contents. The rats were anaesthetized with an overdose of pentobarbital sodium (Narcoren™) and killed by cutting the vena cava caudalis. The abdominal cavity was opened and the diaphragm was cut carefully allowing the lungs to collapse. Heart, oesophagus, upper half of trachea, thymus and lung associated lymph-nodes (LALN) were removed from the lung convolution.

For lung burden analysis and histopathology the lung and the lower half of the trachea were weighed and after putting a tourniquet on the main bronchus to the right lung lobes these lung lobes were cut off at the lung tissue border and used for lung retention measurement. The right lung lobes were weighed and stored in plastic tubes (Greiner®) at -25±5°C. The left lung lobe was inflated under a pressure of 0.2 m H_2O with formalin and fixed by immersion for a minimum of 2 hours, and used for histopathology. Thereafter the weight of the lower part of the trachea was recorded and the weight of the left lung lobe was calculated as difference between total lung including lower part of trachea and the sum of right lung lobes and lower part of trachea.

6.2 Lung Burden Measurement

After sacrifice the right lung lobes were used for fiber retention analysis.

The lung tissue was dissolved by the same procedure which was used in previous studies after inhalation of toner (Muhle et al., 1990).

The lung tissue was placed in polypropylene centrifuge tubes, minced by scissors and digested in 4 ml of 25% tetramethylammonium hydroxide in methanol overnight at 50°C. Next the solution was cooled, diluted with 8 ml of methanol, sonicated for 10 min and then centrifuged for 25 at 39,000 x g. The liquid phase was removed and discarded, and the rinsing procedure was repeated. Next 4 ml freshly prepared 50% HNO_3 (made by mixing 65% HNO_3/Methanol; 10/4; v/v) was added, and the mixture was sonicated and incubated overnight at 50°C. After cooling, 8 ml methanol was added, and the mixture sonicated for 10 min and centrifuged. Again the liquid phase was removed and the rinsing procedure repeated. The remaining methanol was evaporated and the pellet was resuspended in water (containing 0.5% Tween® 80) and filtered on a Nuclepore® filter (25 or 47 mm diameter).

This procedure of lung digesting was validated prior to use. Three untreated lungs were spiked with 0.05, 0.1 or 0.5 mg of the test fibers. The mean fiber number (WHO and long fiber fraction) in the digested lung of each dose level was within 100% ± 25% and the size distribution of fibers recovered was not statistically different from the added test fibers (see Appendix 6). A report on the lung digestion validation was sent to the Study Monitor for approval prior to the digestion of any lungs from this study.

The fiber and particle concentration and the size distribution of fibers and particles were analyzed in SEM as described in Section **3.4**.

From the shape of the fibers and the density (1.0 g/cm^3 assumed for carbon fibers) the mass of the retained fibers was estimated assuming cylindrical geometry. From this data the total number of fibers per lung was calculated for each animal.

At the sacrifice date 15 weeks postexposure also the lung-associated lymph nodes (LALN) were prepared for fiber analysis (see study plan amendment 1 and 5). LALN of all animals per group of that sacrifice date were pooled, and treated and analyzed for translocated fibers using the same procedure as described for lungs.

6.3 Bronchoalveolar Lavage

At 4 days and 7 and 14 weeks post-treatment, a bronchoalveolar lavage (BAL) was performed and the lavage fluid collected for differential cell counts and analysis of biochemical parameters. With minor alterations, the procedure followed the method described by Henderson et al. (1987).

Following preparation, the lungs were lavaged with saline using five lavages of 5 ml each. The lavage fluid was collected separately for the lavages 1 and 2 (first series, without massage of lungs)

and for the lavages 3 to 5 (second series, using mild massage of lungs) in calibrated tubes and the collected volume was recorded. Until processing the lavage fluid was kept on ice.

After centrifugation of the combined lavage fluid of the lavages 1 and 2, biochemical indicators relevant for diagnosis of lung damage were determined in the supernatant (lactic dehydrogenase - LDH, β-glucuronidase, total protein).

The cell pellet of the first lavage series was resuspended in the residual supernatant, combined with the second lavage series and this suspension was used for cell analysis. Leukocyte concentration of the lavagate was determined using a counting chamber and two cytoslides were prepared with a cytocentrifuge (Shandon Co., Frankfurt, Germany) for differential cell count of leukocytes (macrophages, neutrophils, eosinophils, lymphocytes; additionally: epithelial cells). Another aliquot was used for a viability assay of lavaged cells (trypan blue exclusion test).

The justification of the parameters is given below:

Cytological parameters

- total cell count (recruitment of lung leukocytes)
- viability test (giving percentage of alive leukocytes among the total number of cells)
- differential cell count (inflammatory (PMNs) or immunological (lymphocytes) reactions)

Biochemical parameters

- lactic dehydrogenase (LDH = cytosolic marker enzyme; increased permeability of membranes, cell damage and lysis)
- β-glucuronidase (measure of phagolysosomal enzyme of macrophages; lysis of macrophages)
- total protein (marker of transsudation; damage of epithelial cells).

These parameters were analyzed according to routine clinical chemistry protocols using a Cobas Fara device (Roche Co., Grenzach, Germany):

Lactic Dehydrogenase (EC 1.1.1.27, U/l)
Technique: kinetic UV-test at 37°C
References: Bais R., Philnox M (1994) Eur. J. Clin Chem. Clin. Biochem. 32, 639
ß-Glucuronidase (EC 3.2.1.31, U/l)
Technique: colour reaction at 37°C
References: Fishman WH In: Bergmeier HU (ed) Methoden der enzymatischen Analyse, VCH, Weinheim, 1974
Total Protein (mg/l)
Technique: Lowry method
References: In: Bergmeier HU (ed) Methoden der enzymatischen Analyse, VCH, Weinheim, 1974.

6.4 Histopathology

Conventional histopathological examination including fibrosis scoring (H&E staining and Masson-Trichrome staining) was performed on the left lung lobes and trachea (H&E staining only) of animals listed in **Table 2**. The McConnell-Wagner fibrosis scoring system (McConnell et al. 1984) was used. Additionally the EPS evaluation system including scoring for collagen deposition at the bronchiolo-alveolar junction (Annex in ECB/TM/16(97) rev, 1) was used. A quantitative evaluation of fibrosis using a morphometric method according to the criteria from J. Davis, IOM, Edinburgh was also done. The Wagner scoring system is from grade 1 to 8, and grade 4 correspond to evidence of early interstitial fibrosis. The EPS system includes grade 0 to 5 and grade 2 is corresponding to evidence of early interstitial fibrosis.

Formalin-fixed tissue of the terminal bronchioles, pleural cells and lung parenchymal cells were examined for cell proliferation using the sensitive S-phase response method. Proliferating cells were labeled by 5-bromo-2'-deoxyuridine (BrdU) which was administered to the animals by a minipump which was implanted prior to sacrifice. The lung section slides were prepared according to histological routine procedure and stained immunohistochemically following denaturation of the DNA (antibody technique). The slides were evaluated by analyzing an appropriate number of each airway cells and cells of the proximal regions of the pulmonary parenchyma per rat. In addition, pleural cells were analyzed and the unit length labeling index (ULLI) (at least 1 cm in length of pleura per lung section) estimated.

For the terminal bronchioles the Unit Length Labelling Index (ULLI) method was used. The ULLI of at least 4 terminal bronchioli (longitudinally sectioned) was determined. The number of BrdU-positive cells was recorded on a length of approximately 2 x 500 µm of bronchiolar epithelium, counting from the bronchiolo-alveolar transition towards the proximal terminal bronchiole and the number of BrdU-positive cells per mm of airway surface (or basement membrane) was calculated.

For the lung parenchyma four fields (top, below, right and left to the bronchiolus; distance of 1 alveolus between the counting frame and the bronchiolar wall, see **Fig. 2**) per evaluated bronchiolus were analyzed. All cells of the parenchyma were analyzed except cells of larger blood vessels, PMNs and intraalveolar macrophages which had no contact to the alveolar wall. A minimum of 2000 lung parenchymal cells were counted per animal.

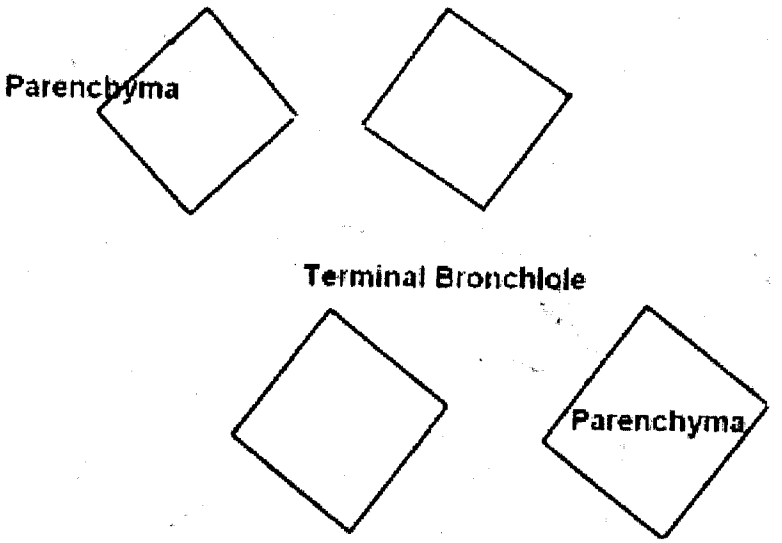

Fig. 2 Regions for analyzing of cell proliferation in lung parenchyma

6.5 Data Collection and Transformation

The data of fiber length and diameter were recorded on personal computers in the laboratory and were transferred to the central computer unit. The analysis of the data was done with the SAS software package (SAS Institute, Cary, NC, USA).

6.6 Evaluation of data

The individual toxicological data was compared to the clean air control group. For numeric parameters the statistics was done by using Dunnett's or Tukey's test. For comparison of histo-pathological findings a pairwise Fisher's exact test between control and treatment groups was performed.

6.7 Transfer of Electronic Data from Fraunhofer ITEM to the Sponsor

On request by the sponsor data which are stored electronically (body weight, lung wet weight, fiber length and diameter data) at Fraunhofer ITEM can be transferred to the sponsor.

Data may be transferred by email or by sending 3.5"-DS,HD Diskettes (1.44 MB) IBM formatted. The

format of the data will be specified by arrangement with the sponsor. After completion of the study the following data will be stored on a CD and sent to the sponsor (i.e. structure: study no., substance/group, animal no., exposure period, recovery period, parameters examined):

Study plan, final report, body weight (g), LDH (U/L), ß-Glucuronidase (U/L), total protein (mg/L), cell concentration (cells/ml), macrophages (%), PMN (%), lymphocytes (%), terminal body weight (g), lung wet weight (g), histopathological findings including semiquantitative assessment of fibrosis (EPS grade, Wagner grade), cell proliferation data (labelling index parenchyma, proliferation index terminal bronchioles, proliferation index pleural cells, fibers/lung, WHO fibers/lung, fibers (L>20μm)/lung, and particles/lung.

7 QUALITY ASSURANCE

The Quality Assurance Unit (QAU) of Fraunhofer ITEM inspected critical phases throughout the study and audited raw data and the reports.

8 STORAGE AND RETENTION OF RECORDS AND MATERIAL

After completion of the study and issuance of the final report, the study plan with amendments and deviations, the original final report, specimens of the test fiber, the samples for scanning electron microscopy, the raw data of all measurements and other material will be transferred to the archives of Fraunhofer ITEM. All materials will be properly indexed and catalogued and stored for the minimum period of time in compliance with GLP principles. The material to be retained is not limited to the list above. No materials will be disposed of without the written consent of the sponsor and Fraunhofer ITEM.

9 RESULTS

9.1 Concentration and Fiber Size Distribution in the Aerosol

The aerosol concentrations were measured by SEM.

The means and standard deviations of the fiber and particle concentrations in the aerosol samples of the three exposure chambers are summarized in **Table 3**. The data for individual days are shown in Appendix 4. The size distribution of the aerosol samples is presented in **Table 4** and **5**. The size distribution of individual days is shown in Appendix 5a.

Table 3 Numerical and gravimetric concentration of fibers in the exposure chambers

Group		Numerical concentration [1/ml]				Mass concentration [mg/m³]		
						Estimation from SEM analysis*		Weighing of filter samples[a]
		Fibers	WHO fibers	Fibers L>20µm	Particles	Fibers	Particles	
Carbon fiber Low	Mean	64.2	48.6	15.0	6.2	1.8	0.1	2.1
	SD	17.3	10.2	3.5	7.2	0.5	0.1	0.7
Carbon fiber Med	Mean	225.0	169.5	50.7	22.1	6.7	0.3	6.7
	SD	50.7	28.1	10.7	25.1	1.6	0.1	0.9
Carbon fiber High	Mean	693.6	531.1	158.3	73.7	21.5	0.9	20.6
	SD	115.2	85.4	27.2	66.1	3.9	0.5	2.5

SD: Standard deviation

* Definition of fibers and particles and method for estimation of mass see **3.4.3**

[a] Gravimetric concentration of fibers + particles

Table 4 Size distribution of aerosol samples (Percentile values)

Group	Fiber length [µm]			Fiber diameter [µm]			Estimated aerodynamic diameter* [µm]		
	10%<	50%<	90%<	10%<	50%<	90%<	10%<	50%<	90%<
Definition: All Fibers (L/D >3)									
Carbon fiber low	3.3	9.9	33.5	0.43	0.90	1.88	3.0	6.0	10.7
Carbon fiber med	3.3	9.8	34.5	0.44	0.92	1.95	3.1	5.9	11.8
Carbon fiber high	3.4	10.0	36.3	0.44	0.92	1.93	3.1	6.0	10.6
Definition: Fibers L> 20 µm									
Carbon fiber low	21.4	31.1	72.6	0.56	1.13	2.34	3.4	6.5	10.8
Carbon fiber med	21.7	31.1	74.4	0.61	1.17	2.51	3.4	6.8	12.1
Carbon fiber high	21.7	32.0	78.4	0.60	1.18	2.44	3.5	6.7	11.2
Definition: WHO-Fibers									
Carbon fiber low	6.2	13.0	37.7	0.50	1.03	1.92	2.8	4.8	7.0
Carbon fiber med	6.2	13.2	39.7	0.53	1.06	2.00	2.9	5.0	7.7
Carbon fiber high	6.3	13.2	41.7	0.52	1.05	1.96	2.9	5.0	7.5

* Method for estimation of aerodynamic diameter see **3.4.3**

Table 5 Size distribution of aerosol samples (Mean values)

Group	Fiber length [µm]				Fiber diameter [µm]			
	Arithmetic		Geometric		Arithmetic		Geometric	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Fraction All fibers (L/D>3)								
Carbon fiber low	16.16	21.05	10.35	2.49	1.07	0.65	0.91	1.77
Carbon fiber med	16.28	20.32	10.37	2.51	1.09	0.67	0.92	1.78
Carbon fiber high	17.13	23.62	10.65	2.54	1.09	0.66	0.92	1.77
Fraction L>20µm								
Carbon fiber low	41.06	31.20	35.17	1.64	1.33	0.79	1.14	1.73
Carbon fiber med	41.14	28.62	35.41	1.63	1.38	0.83	1.19	1.72
Carbon fiber high	43.95	35.34	36.94	1.68	1.39	0.82	1.20	1.71
Fraction WHO								
Carbon fiber low	19.71	21.44	14.49	2.05	1.14	0.56	1.00	1.66
Carbon fiber med	19.94	21.27	14.57	2.06	1.17	0.58	1.04	1.66
Carbon fiber high	20.82	24.39	14.85	2.10	1.16	0.56	1.03	1.65

SD: Standard deviation

9.2 Body Weights

The body weight development is presented in **Fig. 3**. Compared to controls all treatment groups showed a similar body weight increase during the study. The mean values and data of individual animals are listed in Appendix 1.

9.3 Lung Wet Weights and Terminal Body Weights

The lung wet weights were determined of lungs taken for retention and BAL measurements. These data are given together with the terminal body weights in **Table 6**. The individual animal data are listed in Appendix 2a.

The necropsy findings are summarized in Appendix 2b.

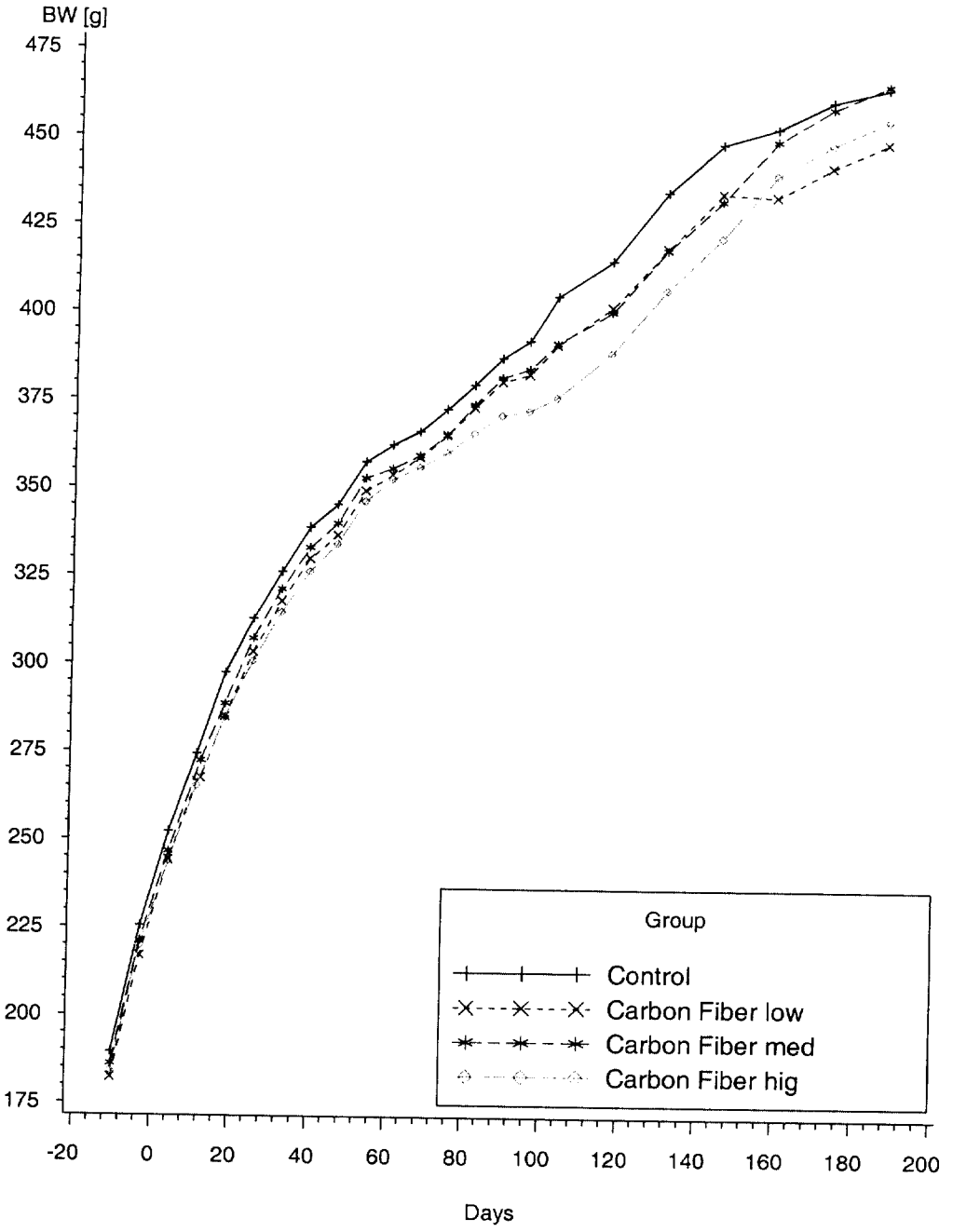

Fig. 3 Body weight development during (day 0-90) and after inhalation (day 91-186)

Table 6 Terminal body and lung wet weights

Group		Terminal body weight [g]		
		Postexposure interval [weeks]		
		1	8	15
Control	Mean	380.2	449.1	461.0
	SD	24.2	42.4	39.4
	N	10	10	11
Carbon fiber low	Mean	380.8	440.4	445.5
	SD	37.5	28.3	26.0
	N	10	10	12
Carbon fiber med	Mean	381.1	423.3	461.9
	SD	21.9	31.9	37.8
	N	10	10	12
Carbon fiber high	Mean	381.7	*407.1	451.3
	SD	31.9	33.0	28.3
	N	10	10	12
Group		Lung wet weight [g]		
		Postexposure interval [weeks]		
		1	8	15
Control	Mean	1.3139	1.3942	1.4615
	SD	0.0726	0.1473	0.1450
	N	10	10	11
Carbon fiber low	Mean	*1.4288	1.4724	1.5210
	SD	0.0660	0.0566	0.0806
	N	10	10	12
Carbon fiber med	Mean	***1.6060	**1.5737	**1.6788
	SD	0.0633	0.0942	0.1691
	N	10	10	12
Carbon fiber high	Mean	***1.7735	***1.6971	***1.8138
	SD	0.1176	0.1546	0.1600
	N	10	10	12

Statistics: Anova + Dunnett's tests. (Two-Sided) * P<=5% ; ** P<=1% ; *** P<=0.1%

SD: Standard deviation N: Number of animals

9.4 Retention of Fibers in the Lungs

The results of fiber retention are summarized in **Table 7**. At 15 weeks postexposure also the fiber content within the pooled lung associated lymph nodes (LALN) was analysed per group. The data for individual animals is listed in Appendix 7. From lung retention data the half-times for the clearance of fibres were calculated (see **Table 8**). The size distribution of fibers in lung and LALN for the different sacrifice dates is shown in **Table 9** for percentile values and in **Table 10** for arithmetic and geometric means. The size distribution of individual animals is shown in Appendix 5b.

Table 7 Analysis of fibers in the lungs and lung-associated lymph nodes (LALN)

Group	Sacrifice after end of exposure [weeks]		Number of Fibers [10^6/lung]					Particles [10^6/lung]	Estimated mass from SEM analysis [µg/lung]*	
			All	L≤5 µm	L=5-20 µm	WHO	L> 20µm		Fibers	Particles
Control	1	Mean	0.0	0.0	0.0	0.0	0.000	0.0	0	0
		SD	0.0	0.0	0.0	0.0	0.001	0.0	0	0
	8	Mean	0.0	0.0	0.0	0.0	0.000	0.0	0	0
		SD	0.0	0.0	0.0	0.0	0.000	0.0	0	0
	15	Mean	0.0	0.0	0.0	0.0	0.000	0.0	0	0
		SD	0.0	0.0	0.0	0.0	0.000	0.0	0	0
	15 LALN	Mean	0.00	0.00	0.00	0.00	0.000	0.00	0.0	0.0
Carbon fiber low	1	Mean	17.9	4.8	9.9	13.1	3.267	5.6	267	28
		SD	3.6	1.8	1.7	2.0	0.452	1.5	27	8
	8	Mean	13.6	2.0	8.5	11.6	3.079	7.1	210	22
		SD	2.7	1.0	2.0	1.7	0.393	8.2	19	16
	15	Mean	8.2	1.0	4.7	7.2	2.512	2.5	149	9
		SD	0.2	0.2	0.3	0.3	0.267	0.2	15	1
	15 LALN	Mean	0.07	0.01	0.06	0.07	0.008	0.00	0.8	0.0
Carbon fiber med	1	Mean	87.6	25.6	51.2	62.0	10.758	31.9	1069	154
		SD	7.3	7.0	4.7	5.2	2.027	6.6	105	40
	8	Mean	48.9	8.6	29.9	40.3	10.411	17.1	687	67
		SD	3.9	1.8	1.4	2.5	1.063	7.1	47	21
	15	Mean	35.7	5.1	21.1	30.7	9.540	9.8	641	56
		SD	3.3	1.6	2.0	2.1	0.480	1.8	89	16
	15 LALN	Mean	0.65	0.19	0.43	0.46	0.039	0.47	4.9	7.4
Carbon fiber high	1	Mean	205.1	59.9	113.7	145.2	31.498	71.1	2815	351
		SD	26.3	19.4	15.0	23.3	11.415	12.5	495	71
	8	Mean	137.4	30.0	79.1	107.4	28.264	45.6	1929	166
		SD	5.8	3.9	4.5	5.9	2.471	8.6	235	34
	15	Mean	111.8	13.9	68.4	97.9	29.428	31.3	2150	182
		SD	7.0	3.9	3.6	4.3	1.907	6.1	296	99
	15 LALN	Mean	2.18	1.08	1.02	1.10	0.078	0.91	9.6	6.7

SD: Standard deviation

* Definition of fibers and particles and method for estimation of mass see **3.4.3**

Table 8 Clearance half-time and 95% confidence limit (95%C.L.) of the elimination of test fibres (calculation from lung retention data listed in **Table 7**)

Group	Half-time in days calculated from			
	Number of fibres Mean (95%C.L.)	Number of WHO fibres Mean (95%C.L.)	Number of fibres (L>20µm) Mean (95%C.L.)	Number of particles Mean (95%C.L.)
Linear regression of log-values				
Carbon fiber Low	88 (69 - 122)	113 (86 - 168)	261 (158 - 765)	86 (46 - 684)
Carbon fiber Med	76 (65 - 90)	97 (84 - 114)	639 (244 - ∞)	57 (44 - 82)
Carbon fiber High	113 (93 - 144)	176 (84 - 114)	>1000 (228 - ∞)	83 (63 - 119)
Nonlinear regression (exponential fit) according to EU protocol ECB/TM/26				
Carbon fiber Low	83 (65 - 100) [1.00]	103 (79 - 127) [1.00]	248 (100 - 397) [0.99]	83 (56 - 110) [0.98]
Carbon fiber Med	75 (61 - 88) [0.99]	99 (82 - 116) [0.99]	478 (0 - 966) [1.00]	57 (45 - 70) [0.96]
Carbon fiber High	134 (95 - 173) [1.00]	264 (128 - 401) [1.00]	>1000 (0 - ∞) [0.99]	83 (58 - 107) [0.97]

[0.xx] R-Square (R-square x 100 is the percentage of the variance which can be explained by the single exponential regression model)

Table 9 Size distribution of fibers (L/D > 3/1) in lungs (Percentile values)

Group	Sacrifice after end of exposure [week]	Fiber length [µm]			Fiber diameter [µm]			Estimated aerodynamic diameter* [µm]		
		10%<	50%<	90%<	10%<	50%<	90%<	10%<	50%<	90%<
Definition: All Fibers										
Carbon fiber low	1	3.3	8.6	28.0	0.51	0.91	1.69	1.1	1.9	3.4
	8	4.3	10.8	30.3	0.51	0.90	1.60	1.1	1.9	3.3
	15	4.6	12.5	36.5	0.48	0.91	1.61	1.1	2.0	3.4
	15 LALN	5.0	8.1	20.2	0.54	0.93	1.46	1.2	1.9	2.9
Carbon fiber med	1	3.1	7.7	22.9	0.49	0.89	1.64	1.0	1.8	3.3
	8	3.9	11.0	28.6	0.44	0.85	1.56	1.0	1.9	3.3
	15	4.3	11.8	34.0	0.51	0.91	1.62	1.1	2.0	3.5
	15 LALN	3.7	6.6	15.9	0.45	0.83	1.50	1.0	1.7	2.9
Carbon fiber high	1	2.9	8.1	24.6	0.46	0.89	1.65	1.0	1.8	3.3
	8	3.4	9.9	29.2	0.45	0.86	1.55	1.0	1.9	3.2
	15	4.5	11.8	32.3	0.48	0.94	1.76	1.1	2.0	3.7
	15 LALN	3.2	5.0	10.7	0.44	0.75	1.31	0.9	1.5	2.4
Definition: Fibers L > 20 µm										
Carbon fiber low	1	21.8	28.6	49.1	0.63	1.07	1.78	1.6	2.6	4.0
	8	21.4	28.7	50.0	0.58	1.00	1.66	1.5	2.5	3.8
	15	21.5	30.0	50.6	0.58	1.01	1.76	1.5	2.5	4.0
	15 LALN	22.5	25.7	36.9	0.64	0.92	1.54	1.6	2.2	3.7
Carbon fiber med	1	21.8	28.5	45.8	0.62	1.00	1.78	1.6	2.5	4.1
	8	21.2	28.1	47.5	0.51	0.94	1.75	1.3	2.3	4.0
	15	21.7	30.1	49.3	0.59	1.04	1.75	1.5	2.5	4.0
	15 LALN	20.4	23.5	36.1	0.61	1.07	1.65	1.5	2.5	3.8
Carbon fiber high	1	21.3	27.4	45.5	0.56	1.04	1.85	1.5	2.5	4.3
	8	21.4	29.2	52.1	0.52	0.93	1.73	1.4	2.3	3.9
	15	21.7	28.9	49.2	0.54	1.02	1.88	1.4	2.5	4.3
	15 LALN	20.4	24.6	34.3	0.45	0.84	1.05	1.2	2.1	2.4
Definition: WHO Fibers										
Carbon fiber low	1	5.8	11.5	31.2	0.59	1.03	1.79	1.3	2.2	3.6
	8	6.4	12.4	31.9	0.54	0.94	1.64	1.2	2.1	3.4
	15	6.7	14.4	38.5	0.53	0.96	1.65	1.3	2.2	3.5
	15 LALN	5.7	8.3	22.5	0.55	0.94	1.52	1.3	2.0	2.9
Carbon fiber med	1	5.8	10.7	26.9	0.58	1.01	1.75	1.3	2.2	3.5
	8	6.2	13.1	31.1	0.48	0.92	1.64	1.1	2.0	3.4
	15	6.5	13.8	36.1	0.56	0.97	1.67	1.3	2.2	3.6
	15 LALN	5.3	7.4	18.5	0.49	0.87	1.62	1.1	1.8	3.1
Carbon fiber high	1	6.0	11.0	27.9	0.54	1.02	1.79	1.3	2.2	3.6
	8	6.1	12.4	32.3	0.49	0.95	1.61	1.2	2.1	3.4
	15	6.4	13.4	34.1	0.52	0.99	1.81	1.2	2.2	3.8
	15 LALN	5.3	6.6	17.2	0.51	0.88	1.44	1.2	1.9	2.7

* Method for estimation of aerodynamic diameter see **3.4.3**

Table 10 Size distribution of fibers (L/D > 3/1) in lungs (Mean values)

Group	Sacrifice after end of exposure [week]	Fiber length [µm]				Fiber diameter [µm]			
		Arithmetic		Geometric		Arithmetic		Geometric	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Fraction L/D>3									
Carbon fiber low	1	12.65	11.76	9.07	2.23	1.02	0.50	0.92	1.60
	8	14.85	12.38	11.26	2.10	0.99	0.47	0.89	1.56
	15	17.11	13.95	12.75	2.18	1.00	0.47	0.90	1.59
	15 LALN	10.81	7.08	9.12	1.76	1.00	0.39	0.92	1.48
Carbon fiber med	1	10.92	10.01	8.09	2.12	0.99	0.48	0.89	1.59
	8	14.23	11.46	10.76	2.13	0.95	0.47	0.84	1.63
	15	16.09	13.27	11.99	2.17	1.01	0.49	0.91	1.58
	15 LALN	8.19	6.03	6.90	1.73	0.91	0.43	0.82	1.57
Carbon fiber high	1	11.46	10.48	8.33	2.21	0.99	0.52	0.88	1.65
	8	13.77	12.55	9.92	2.25	0.95	0.47	0.84	1.63
	15	15.97	13.47	12.05	2.12	1.05	0.53	0.93	1.62
	15 LALN	6.50	4.87	5.54	1.67	0.81	0.34	0.75	1.51
Fraction L>20									
Carbon fiber low	1	32.64	12.71	30.78	1.38	1.15	0.49	1.05	1.52
	8	32.71	13.25	30.78	1.39	1.09	0.49	1.00	1.51
	15	34.04	13.31	32.01	1.40	1.10	0.49	1.01	1.53
	15 LALN	27.40	6.17	26.79	1.23	1.02	0.42	0.95	1.46
Carbon fiber med	1	32.28	12.51	30.51	1.37	1.13	0.52	1.03	1.51
	8	31.83	11.81	30.08	1.37	1.05	0.50	0.94	1.58
	15	33.67	13.10	31.71	1.39	1.12	0.47	1.03	1.51
	15 LALN	26.39	8.42	25.47	1.28	1.13	0.43	1.04	1.49
Carbon fiber high	1	31.44	12.31	29.68	1.38	1.14	0.55	1.03	1.59
	8	33.41	13.95	31.22	1.41	1.05	0.52	0.95	1.58
	15	33.40	14.57	31.24	1.40	1.15	0.56	1.03	1.60
	15 LALN	26.21	5.48	25.67	1.23	0.79	0.25	0.75	1.39
Fraction WHO									
Carbon fiber low	1	15.78	12.17	12.67	1.88	1.12	0.48	1.03	1.53
	8	16.60	12.39	13.52	1.85	1.02	0.44	0.94	1.53
	15	18.99	13.90	15.24	1.91	1.04	0.45	0.94	1.55
	15 LALN	11.42	7.04	9.90	1.66	1.02	0.39	0.95	1.46
Carbon fiber med	1	13.90	10.49	11.48	1.78	1.10	0.47	1.01	1.53
	8	16.47	11.42	13.65	1.81	1.00	0.46	0.90	1.59
	15	18.11	13.28	14.60	1.90	1.05	0.46	0.96	1.53
	15 LALN	9.90	6.42	8.66	1.61	0.96	0.43	0.87	1.54
Carbon fiber high	1	14.67	10.92	12.05	1.81	1.11	0.50	1.00	1.58
	8	16.59	12.81	13.34	1.88	1.02	0.46	0.92	1.59
	15	17.65	13.46	14.25	1.88	1.09	0.50	0.98	1.59
	15 LALN	9.06	5.76	7.99	1.57	0.95	0.37	0.88	1.48

SD: Standard deviation

9.5 Bronchoalveolar Lavage

During the recovery period bronchoalveolar lavages (BAL) were performed on 5 rats per group and sacrifice date (at the last sacrifice date 6 rats for the control group and 7 rats for treatment group were used). The following biochemical parameters were measured in the supernatant of the bronchoalveolar lavagate: lactic dehydrogenase (LDH), β-glucuronidase (βGL) and total protein.

The results are summarized in **Table 11** and in Appendix 3a.

The results of the differential cell count are presented in **Table 12**, Appendix 3b and **Fig. 4.**

Table 11 Biochemical parameters in the BAL fluid

Group		1 week post-exposure			8 weeks post-exposure			15 weeks post-exposure		
		LDH	βGL	Protein	LDH	βGL	Protein	LDH	βGL	Protein
		U/l	U/l	mg/l	U/l	U/l	mg/l	U/l	U/l	mg/l
Control	Mean	38	0.2	99	34	0.3	105	33	0.2	103
	SD	8	0.1	10	3	0.1	10	10	0.1	16
	N	5	5	5	5	5	5	6	6	6
Carbon fiber low	Mean	67	0.3	**152	46	0.3	141	51	0.1	133
	SD	22	0.1	13	9	0.1	30	17	0.0	20
	N	5	5	5	5	5	5	7	7	7
Carbon fiber med	Mean	90	0.4	***182	66	0.3	***172	76	0.2	***179
	SD	39	0.1	34	11	0.1	19	55	0.1	24
	N	5	5	5	5	5	5	7	7	7
Carbon fiber high	Mean	***143	***0.7	***204	***122	0.3	***216	*109	0.3	***206
	SD	52	0.1	16	49	0.2	25	85	0.1	30
	N	5	5	5	5	5	5	7	7	7

Statistics: Anova + Dunnett's tests. (Two-Sided) * P<=5% ; ** P<=1% ; *** P<=0.1%

SD: Standard deviation N: Number of animals

Table 12 Cell concentration and percentage of cells in the BAL fluid

Group		Postexposure interval [weeks]						
		1						
		Cell concentration [cells/ml]	Macropages [%]	Eosinophil PMNs [%]	Neutrophil PMNs [%]	Lymphocytes [%]	Cell Viability	Epithelial cells per 200 cells
Control	Mean	143250	98.8	0.0	0.8	0.5	99	3.2
	SD	41567	1.4	0.0	0.9	0.6	0	2.2
	N	5	5	5	5	5	5	5
Carbon fiber low	Mean	148500	95.2	0.1	3.0	1.8	100	4.0
	SD	6697	1.6	0.1	1.2	1.3	1	2.0
	N	5	5	5	5	5	5	5
Carbon fiber med	Mean	168750	***84.5	0.0	***8.7	**6.9	99	3.6
	SD	47145	2.5	0.0	0.5	2.4	1	2.1
	N	5	5	5	5	5	5	5
Carbon fiber high	Mean	162750	***80.4	0.0	***11.1	**8.6	99	4.8
	SD	33018	7.4	0.0	4.1	5.0	2	2.0
	N	5	5	5	5	5	5	5
Group		Postexposure interval [weeks]						
		8						
		Cell concentration [cells/ml]	Macropages [%]	Eosinophil PMNs [%]	Neutrophil PMNs [%]	Lymphocytes [%]	Cell Viability	Epithelial cells per 200 cells
Control	Mean	144000	99.5	0.0	0.3	0.3	98	3.0
	SD	13561	0.3	0.0	0.1	0.3	1	0.7
	N	5	5	5	5	5	5	5
Carbon fiber low	Mean	145000	97.3	0.1	1.5	1.2	99	3.4
	SD	27114	0.9	0.1	0.8	0.1	1	1.8
	N	5	5	5	5	5	5	5
Carbon fiber med	Mean	176250	**90.9	0.0	**5.4	*3.8	99	2.8
	SD	45303	4.7	0.0	4.0	3.1	2	2.5
	N	5	5	5	5	5	5	5
Carbon fiber high	Mean	*202500	**90.8	0.1	*5.3	*3.9	99	3.0
	SD	34267	4.1	0.1	2.3	1.9	1	1.6
	N	5	5	5	5	5	5	5

Statistics: Anova + Dunnett's tests. (Two-Sided) * P<=5% ; ** P<=1% ; *** P<=0.1%

SD: Standard deviation N: Number of animals

Table 12 (cont.) Cell concentration and percentage of cells in the BAL fluid

Group		Postexposure interval [weeks]						
		15						
		Cell concentration [cells/ml]	Macropages [%]	Eosinophil PMNs [%]	Neutrophil PMNs [%]	Lymphocytes [%]	Cell Viability	Epithelial cells per 200 cells
Control	Mean	155000	98.8	0.0	0.5	0.8	99	7.3
	SD	22638	0.6	0.0	0.4	0.4	1	2.1
	N	6	6	6	6	6	6	6
Carbon fiber low	Mean	155000	96.9	0.0	2.1	1.0	99	3.6
	SD	39804	0.6	0.1	0.6	0.6	1	0.8
	N	7	7	7	7	7	7	7
Carbon fiber med	Mean	180536	**93.6	0.0	*3.6	**2.8	99	3.7
	SD	41975	1.8	0.0	1.4	1.3	1	1.6
	N	7	7	7	7	7	7	7
Carbon fiber high	Mean	194643	***90.8	0.0	***5.7	***3.5	99	3.1
	SD	48567	4.2	0.0	3.6	1.1	1	2.0
	N	7	7	7	7	7	7	7

Statistics: Anova + Dunnett's tests. (Two-Sided) * P<=5% ; ** P<=1% ; *** P<=0.1%

SD: Standard deviation N: Number of animals

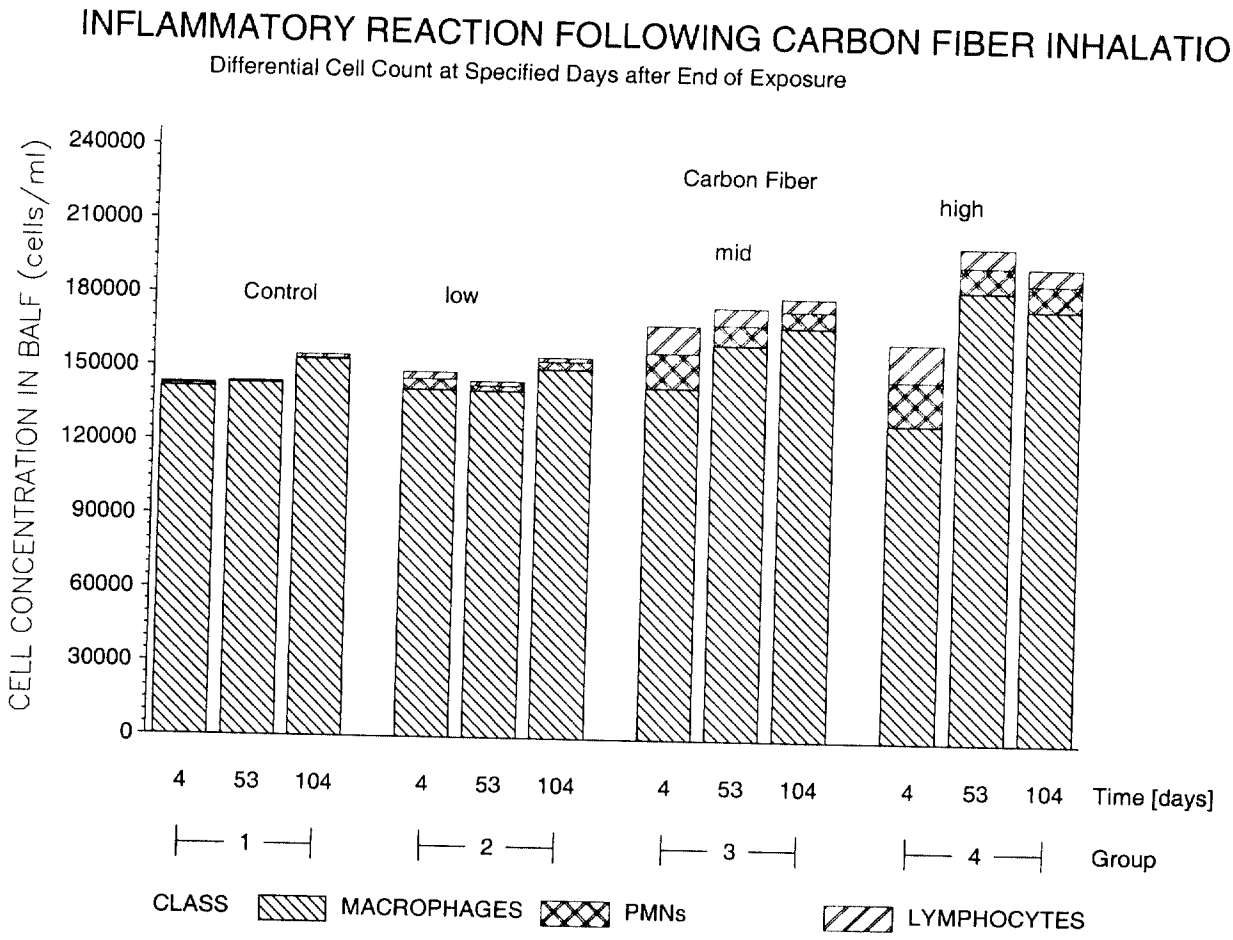

Fig. 4 Differential cell counts in BAL fluids at specified dates after end of exposure

9.6 Histopathology

9.6.1 Histopathological Findings

The results of the histopathological examination after 3 months of inhalation and 5 days postexposure period are summarized in **Table 13**. The results after a 8-week post-exposure period and a 15-week post-exposure period are also given in **Table 13**. Some light micrographs of the lungs of animals of the three treatment groups of the 15-week post-exposure sacrifice are presented in Appendix 9

The results of fibrosis evaluation are presented in **Table 14**

The individual animal data are shown in Appendix 8.

Results of the 5-days postexposure subgroup

Trachea:

Single males of the medium (no. 030003) and high (no. 040005) dose groups, respectively, showed a very slight (minimal) (multi)focal hyperplasia of mucous (goblet) cells within the epithelial lining of the trachea.

Lungs:

No relevant lesions (corresponding to Wagner grade 1 and EPS grade 0) were observed in the clean air control animals (group 01). The only finding in this group was a very slight focal alveolar histiocytosis in animal no. 010002.

All rats of the fiber inhalation groups (5/5, each group) had dose-dependent multifocal very slight (minimal) to moderate alveolar and interstitial accumulation of fiber-containing macrophages. A very slight degree of macrophage accumulation was observed in 5/5 animals of the low dose group (group 02) while all rats (5/5) of the medium dose group (group 03) showed slight accumulation of fiber-laden macrophages. The high dose group (group 04) had both slight (1/5) and moderate (4/5) degrees of fiber-associated macrophage accumulation. The fiber-laden macrophages were preferentially located in the alveolar duct region, either within alveoli or within the peribronchiolar/-interalveolar interstitium. In addition, 2/5 and all (5/5) males of the medium and high dose groups, respectively, showed very slight (group 03: 2/5; group 04: 1/5) to slight (group 04: 4/5) numbers of multinucleated giant cells (macrophages) which were associated with interstitial deposits of fibers.

Dose-dependent microgranulomas (foci of fiber-associated granulomatous inflammation) were seen in 5/5 rats of all fiber-exposed groups at very slight (group 02: 5/5), slight (group 03: 5/5; group 04: 3/5) to moderate (group 04: 2/5) degrees.

Another change which occurred dose-dependently at very slight (group 02: 4/5) to slight (group 02: 1/5; groups 03 and 04: 5/5 each) degrees in all animals of the fiber exposure groups was multifocal bronchiolo-alveolar hyperplasia, mainly of the bronchiolar type (alveolar bronchiolization). The

hyperplastic epithelium developed in association with inflammatory and fibrotic foci within the alveolar duct region and was usually continuous with the epithelium of the terminal bronchioles. In addition, 2/5 and 5/5 rats of the medium and high dose groups, respectively, revealed very slight (group 03: 1/5; group 04: 2/5) to slight (group 03: 1/5; group 04: 3/5) (multi)focal mucous (goblet) cell hyperplasia of the bronchiolar epithelium.

All rats of the fiber exposure groups showed dose-dependent very slight to slight multifocal interstitial fibrosis which mainly affected the bronchiolo-alveolar junctions. Interstitial fibrosis of slight degree (corresponding to Wagner-McConnell grade 4 and to EPS grade 2) was diagnosed in 2/5 and 5/5 rats of groups 03 and 04, respectively, while all (5/5) animals of group 02 and 3/5 rats of group 03 showed very slight (minimal) interstitial fibrosis (corresponding to Wagner-McConnell grades 3 and to EPS grade 1). Fibrosis of slight degree was also quantitatively evaluated according to the proposal of McConnell and Davis, the results are given in **Table 14**. EPS grade 2 and Wagner grade 4 correspond to evidence of early interstitial fibrosis.

Results of the 8-weeks postexposure subgroup

Trachea:

In none of the animals abnormalities were detected in the trachea.

Lungs:

No relevant lesions (corresponding to Wagner grade 1 and EPS grade 0) were observed in the clean air control animals (group 01).

All rats of the fiber inhalation groups (5/5, each group) had dose-dependent multifocal very slight (minimal) to moderate alveolar and interstitial accumulation of fiber-containing macrophages. A very slight (4/5 rats) to slight (1/5 rats) degree of macrophage accumulation was observed in the low dose group (group 02) while all rats (5/5) of the medium dose group (group 03) showed slight accumulation of fiber-laden macrophages. The high dose group (group 04) had both slight (2/5) and moderate (3/5) degrees of fiber-associated macrophage accumulation. The fiber-laden macrophages were preferentially located in the alveolar duct region, either within alveoli or within the peribronchiolar/interalveolar interstitium. In addition, 1/5 and 5/5 males of the low and medium dose groups, respectively, showed very slight and all (5/5) males of the high dose group slight numbers of multinucleated giant cells (macrophages) which were associated with interstitial deposits of fibers.

Dose-dependent microgranulomas (foci of fiber-associated granulomatous inflammation) were seen in 5/5 rats of all fiber-exposed groups at very slight (group 02: 4/5), slight (group 02: 1/5; group 03: 5/5; group 04: 3/5) to moderate (group 04: 2/5) degrees.

Another change which occurred dose-dependently at very slight (group 02: 3/5) to slight (group 02: 2/5; groups 03 and 04: 5/5 each) degrees in all animals of the fiber exposure groups was multifocal bronchiolo-alveolar hyperplasia, mainly of the bronchiolar type (alveolar bronchiolization). The

hyperplastic epithelium developed in association with inflammatory and fibrotic foci within the alveolar duct region and was usually continuous with the epithelium of the terminal bronchioles. In addition, 4/5 rats of the high dose groups, revealed very slight (group 04: 3/5) to slight (group 04: 1/5) (multi)focal mucous (goblet) cell hyperplasia of the bronchiolar epithelium.

All rats of the fiber exposure groups showed dose-dependent very slight to slight multifocal interstitial fibrosis which mainly affected the bronchiolo-alveolar junctions. Interstitial fibrosis of slight degree (corresponding to Wagner-McConnell grade 4 and to EPS grade 2) was diagnosed in 1/5 and 5/5 rats of groups 03 and 04, respectively, while all (5/5) animals of group 02 and 4/5 rats of group 03 showed very slight (minimal) interstitial fibrosis (corresponding to Wagner-McConnell grades 3 and to EPS grade 1). Fibrosis of slight degree was also quantitatively evaluated according to the proposal of McConnell and Davis, the results are given in **Table 14**. EPS grade 2 and Wagner grade 4 correspond to evidence of early interstitial fibrosis.

Results of the 15-weeks postexposure subgroup

Trachea:

A single male of the medium dose group (no. 030013) showed slight (multi)focal hyperplasia of mucous (goblet) cells within the epithelial lining of the trachea.

Lungs:

No relevant lesions (corresponding to Wagner grade 1 and EPS grade 0) were observed in the clean air control animals (group 01).

All rats of the fiber inhalation groups (5/5, each group) had dose-dependent multifocal very slight (minimal) to moderate alveolar and interstitial accumulation of fiber-containing macrophages. A very slight degree of macrophage accumulation was observed in 5/5 animals of the low dose group (group 02) while all rats (5/5) of the medium dose group (group 03) showed slight accumulation of fiber-laden macrophages. The high dose group (group 04) had both slight (1/5) and moderate (4/5) degrees of fiber-associated macrophage accumulation. The fiber-laden macrophages were preferentially located in the alveolar duct region, either within alveoli or within the peribronchiolar/-interalveolar interstitium. In addition, 1/5 and 3/5 males of the low and medium dose groups, respectively, showed very slight and 2/5 and all (5/5) males of the medium and high dose groups, respectively, slight numbers of multinucleated giant cells (macrophages) which were associated with interstitial deposits of fibers. Dose-dependent microgranulomas (foci of fiber-associated granulomatous inflammation) were seen in 5/5 rats of all fiber-exposed groups at very slight (group 02: 5/5), slight (group 03: 5/5; group 04: 3/5) to moderate (group 04: 2/5) degrees.

Another change which occurred dose-dependently at very slight (group 02: 3/5; group 03: 1/5) to slight (group 02: 1/5; group 03: 4/5; group 04: 5/5) degrees in all animals of the fiber exposure groups (except one rat of the low dose group) was multifocal bronchiolo-alveolar hyperplasia, mainly

of the bronchiolar type (alveolar bronchiolization). The hyperplastic epithelium developed in association with inflammatory and fibrotic foci within the alveolar duct region and was usually continuous with the epithelium of the terminal bronchioles. In addition, 2/5 rats of the medium and high dose groups each revealed very slight (group 03: 1/5; group 04: 2/5) to slight (group 03: 1/5) (multi)focal mucous (goblet) cell hyperplasia of the bronchiolar epithelium.

All rats of the fiber exposure groups (except two rats of the low dose group) showed dose-dependent very slight to slight multifocal interstitial fibrosis which mainly affected the bronchiolo-alveolar junctions. Interstitial fibrosis of slight degree (corresponding to Wagner-McConnell grade 4 and to EPS grade 2) was diagnosed in 1/5 and 5/5 rats of groups 03 and 04, respectively, while 3/5 animals of group 02 and 4/5 rats of group 03 showed very slight (minimal) interstitial fibrosis (corresponding to Wagner-McConnell grades 3 and to EPS grade 1). In addition, 2/5 rats of the low dose group without multifocal interstitial fibrosis had Wagner-McConnell grades 3 and EPS grade 1. Fibrosis of slight degree was also quantitatively evaluated according to the proposal of McConnell and Davis, the results are given in **Table 14**. EPS grade 2 and Wagner grade 4 correspond to evidence of early interstitial fibrosis.

Table 13 Histopathological findings

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Summary of Histopathological Findings, 5-days postexposure subgroup

LESIONS	TREATMENT	INCIDENCE OF LESIONS (PERCE! Males			
		Clean Air	~15 F/ml	~50 F/ml	~150 F/ml
TRACHEA					
		(5)	(5)	(4)	(5)
No abnormality detected		5 (100%)	5 (100%)	3 (75%)	4 (80%)
Mucous (goblet) cell hyperplasia very slight		0 (0%)	0 (0%)	1 (25%)	1 (20%)
Score Expanded Totals		0 (0%)	0 (0%)	1 (25%)	1 (20%)
LUNGS					
		(5)	(5)	(5)	(5)
Accumulation of fibre-laden macrophages very slight		0 (0%)	5** (100%)	0 (0%)	0 (0%)
slight		0 (0%)	0 (0%)	5** (100%)	1 (20%)
moderate		0 (0%)	0 (0%)	0 (0%)	4* (80%)
Score Expanded Totals		0 (0%)	5** (100%)	5** (100%)	5** (100%)
Microgranuloma(s) very slight		0 (0%)	5** (100%)	0 (0%)	0 (0%)
slight		0 (0%)	0 (0%)	5** (100%)	3 (60%)
moderate		0 (0%)	0 (0%)	0 (0%)	2 (40%)
Score Expanded Totals		0 (0%)	5** (100%)	5** (100%)	5** (100%)
Interstitial multinucleated giant-cells very slight		0 (0%)	0 (0%)	2 (40%)	1 (20%)
slight		0 (0%)	0 (0%)	0 (0%)	4* (80%)
Score Expanded Totals		0 (0%)	0 (0%)	2 (40%)	5** (100%)

Significance of difference in a pairwise Fisher's test between control and treatment groups: *P<0.05,**P<0.01,***P<0.001
Figures in brackets represent the number of animals from which this tissue was examined microscopically

*** Continued

Table 13 (cont.) Histopathological findings

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Summary of Histopathological Findings, 5-days postexposure subgroup

LESIONS	TREATMENT	INCIDENCE OF LESIONS (PERCE! Males Clean Air	!-15 F/ml	!-50 F/ml	!-150 F/ml
LUNGS		(5)	(5)	(5)	(5)
Bronchiolo-alveolar hyperplasia very slight		0 (0%)	4* (80%)	0 (0%)	0 (0%)
slight		0 (0%)	1 (20%)	5** (100%)	5** (100%)
Score Expanded Totals		0 (0%)	5** (100%)	5** (100%)	5** (100%)
Bronchiolar mucous (goblet) cell hyperplasia very slight		0 (0%)	0 (0%)	1 (20%)	2 (40%)
slight		0 (0%)	0 (0%)	1 (20%)	3 (60%)
Score Expanded Totals		0 (0%)	0 (0%)	2 (40%)	5** (100%)
Interstitial fibrosis very slight		0 (0%)	5** (100%)	3 (60%)	0 (0%)
slight		0 (0%)	0 (0%)	2 (40%)	5** (100%)
Score Expanded Totals		0 (0%)	5** (100%)	5** (100%)	5** (100%)
Alveolar histiocytosis very slight		1 (20%)	0 (0%)	0 (0%)	0 (0%)
Score Expanded Totals		1 (20%)	0 (0%)	0 (0%)	0 (0%)
Wagner grade 1		5 (100%)	0** (0%)	0** (0%)	0** (0%)
Wagner grade 3		0 (0%)	5** (100%)	3 (60%)	0 (0%)
Wagner grade 4		0 (0%)	0 (0%)	2 (40%)	5** (100%)
EPS grade 0		5 (100%)	0** (0%)	0** (0%)	0** (0%)
EPS grade 1		0 (0%)	5** (100%)	3 (60%)	0 (0%)

Significance of difference in a pairwise Fisher's test between control and treatment groups: *P<0.05,**P<0.01,***P<0.001
Figures in brackets represent the number of animals from which this tissue was examined microscopically

*** Continued

Table 13 (cont.) Histopathological findings

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Summary of Histopathological Findings, 5-days postexposure subgroup

LESIONS	TREATMENT	INCIDENCE OF LESIONS (PERCE! Males			
		Clean Air	~15 F/ml	~50 F/ml	~150 F/ml
LUNGS					
EPS grade 2		(5) 0 (0%)	(5) 0 (0%)	(5) 2 (40%)	(5) 5** (100%)

Significance of difference in a pairwise Fisher's test between control and treatment groups: *P<0.05,**P<0.01,***P<0.001
Figures in brackets represent the number of animals from which this tissue was examined microscopically

*** Listing Complete ***

Table 13 (cont.) Histopathological findings

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Summary of Histopathological Findings, 8 weeks postexposure subgroup

LESIONS	TREATMENT	INCIDENCE OF LESIONS (PERCE			
		Males			
		Clean Air	~15 F/ml	~50 F/ml	~150 F/ml
TRACHEA					
No abnormality detected		(5)	(5)	(5)	(5)
		5 (100%)	5 (100%)	5 (100%)	5 (100%)
LUNGS					
		(5)	(5)	(5)	(5)
Accumulation of fibre-laden macrophages					
very slight		0 (0%)	4* (80%)	0 (0%)	0 (0%)
slight		0 (0%)	1 (20%)	5** (100%)	2 (40%)
moderate		0 (0%)	0 (0%)	0 (0%)	3 (60%)
Score Expanded Totals		0 (0%)	5** (100%)	5** (100%)	5** (100%)
Microgranulomas					
very slight		0 (0%)	4* (80%)	0 (0%)	0 (0%)
slight		0 (0%)	1 (20%)	5** (100%)	3 (60%)
moderate		0 (0%)	0 (0%)	0 (0%)	2 (40%)
Score Expanded Totals		0 (0%)	5** (100%)	5** (100%)	5** (100%)
Interstitial multinucleated giant-cells					
very slight		0 (0%)	1 (20%)	5** (100%)	0 (0%)
slight		0 (0%)	0 (0%)	0 (0%)	5** (100%)
Score Expanded Totals		0 (0%)	1 (20%)	5** (100%)	5** (100%)
Bronchiolo-alveolar hyperplasia					
very slight		0 (0%)	3 (60%)	0 (0%)	0 (0%)
slight		0 (0%)	2 (40%)	5** (100%)	5** (100%)
Score Expanded Totals		0 (0%)	5** (100%)	5** (100%)	5** (100%)

Significance of difference in a pairwise Fisher's test between control and treatment groups: *P<0.05,**P<0.01,***P<0.001
Figures in brackets represent the number of animals from which this tissue was examined microscopically

*** Continued

Table 13 (cont.) Histopathological findings

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Summary of Histopathological Findings, 8 weeks postexposure subgroup

LESIONS	TREATMENT	INCIDENCE OF LESIONS (PERCE! Males			
		Clean Air	~15 F/ml	~50 F/ml	~150 F/ml
LUNGS		(5)	(5)	(5)	(5)
Bronchiolar mucous (goblet) cell hyperplasia					
very slight		0 (0%)	0 (0%)	0 (0%)	3 (60%)
slight		0 (0%)	0 (0%)	0 (0%)	1 (20%)
Score Expanded Totals		0 (0%)	0 (0%)	0 (0%)	4* (80%)
Mucous (goblet) cell metaplasia					
very slight		0 (0%)	0 (0%)	1 (20%)	2 (40%)
slight		0 (0%)	0 (0%)	1 (20%)	2 (40%)
Score Expanded Totals		0 (0%)	0 (0%)	2 (40%)	4* (80%)
Interstitial fibrosis					
very slight		0 (0%)	5** (100%)	4* (80%)	0 (0%)
slight		0 (0%)	0 (0%)	1 (20%)	5** (100%)
Score Expanded Totals		0 (0%)	5** (100%)	5** (100%)	5** (100%)
Alveolar inflammatory cell infiltration					
slight		0 (0%)	1 (20%)	0 (0%)	0 (0%)
Score Expanded Totals		0 (0%)	1 (20%)	0 (0%)	0 (0%)
Wagner grade 1		5 (100%)	0** (0%)	0** (0%)	0** (0%)
Wagner grade 3		0 (0%)	5** (100%)	4* (80%)	0 (0%)
Wagner grade 4		0 (0%)	0 (0%)	1 (20%)	5** (100%)
EPS grade 0		5 (100%)	0** (0%)	0** (0%)	0** (0%)
EPS grade 1		0 (0%)	5** (100%)	4* (80%)	0 (0%)

Significance of difference in a pairwise Fisher's test between control and treatment groups: *P<0.05,**P<0.01,***P<0.001
Figures in brackets represent the number of animals from which this tissue was examined microscopically

*** Continued

Table 13 (cont.) Histopathological findings

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Summary of Histopathological Findings, 8 weeks postexposure subgroup

LESIONS	TREATMENT	INCIDENCE OF LESIONS (PERCE) Males			
		Clean Air	~15 F/ml	~50 F/ml	~150 F/ml
LUNGS					
EPS grade 2		(5) 0 (0%)	(5) 0 (0%)	(5) 1 (20%)	(5) 5** (100%)

Significance of difference in a pairwise Fisher's test between control and treatment groups: *P<0.05,**P<0.01,***P<0.001
Figures in brackets represent the number of animals from which this tissue was examined microscopically

*** Listing Complete ***

Table 13 (cont.) Histopathological findings

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Summary of Histopathological Findings, 15 weeks postexposure subgroup

LESIONS	TREATMENT	INCIDENCE OF LESIONS (PERCE) Males			
		Clean Air	~15 F/ml	~50 F/ml	~150 F/ml
TRACHEA					
No abnormality detected		(5)	(5)	(5)	(5)
		5 (100%)	5 (100%)	4 (80%)	5 (100%)
Mucous (goblet) cell hyperplasia slight		0 (0%)	0 (0%)	1 (20%)	0 (0%)
Score Expanded Totals		0 (0%)	0 (0%)	1 (20%)	0 (0%)
LUNGS					
Accumulation of fibre-laden macrophages very slight		(5)	(5)	(5)	(5)
		0 (0%)	5** (100%)	0 (0%)	0 (0%)
slight		0 (0%)	0 (0%)	5** (100%)	1 (20%)
moderate		0 (0%)	0 (0%)	0 (0%)	4* (80%)
Score Expanded Totals		0 (0%)	5** (100%)	5** (100%)	5** (100%)
Microgranulomas very slight		(5)	(5)	(5)	(5)
		0 (0%)	5** (100%)	0 (0%)	0 (0%)
slight		0 (0%)	0 (0%)	5** (100%)	3 (60%)
moderate		0 (0%)	0 (0%)	0 (0%)	2 (40%)
Score Expanded Totals		0 (0%)	5** (100%)	5** (100%)	5** (100%)
Interstitial multinucleated giant-cells very slight		(5)	(5)	(5)	(5)
		0 (0%)	1 (20%)	3 (60%)	0 (0%)
slight		0 (0%)	0 (0%)	2 (40%)	5** (100%)
Score Expanded Totals		0 (0%)	1 (20%)	5** (100%)	5** (100%)

Significance of difference in a pairwise Fisher's test between control and treatment groups: *P<0.05,**P<0.01,***P<0.001
Figures in brackets represent the number of animals from which this tissue was examined microscopically

*** Continued

Table 13 (cont.) Histopathological findings

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Summary of Histopathological Findings, 15 weeks postexposure subgroup

LESIONS	TREATMENT	INCIDENCE OF LESIONS (PERCE! Males			
		Clean Air	~15 F/ml	~50 F/ml	~150 F/ml
LUNGS		(5)	(5)	(5)	(5)
Bronchiolo-alveolar hyperplasia					
very slight		0 (0%)	3 (60%)	1 (20%)	0 (0%)
slight		0 (0%)	1 (20%)	4* (80%)	5** (100%)
Score Expanded Totals		0 (0%)	4* (80%)	5** (100%)	5** (100%)
Bronchiolar mucous (goblet) cell hyperplasia					
very slight		0 (0%)	0 (0%)	1 (20%)	2 (40%)
slight		0 (0%)	0 (0%)	1 (20%)	0 (0%)
Score Expanded Totals		0 (0%)	0 (0%)	2 (40%)	2 (40%)
Mucous (goblet) cell metaplasia					
very slight		0 (0%)	0 (0%)	2 (40%)	3 (60%)
slight		0 (0%)	0 (0%)	0 (0%)	1 (20%)
Score Expanded Totals		0 (0%)	0 (0%)	2 (40%)	4* (80%)
Interstitial fibrosis					
very slight		0 (0%)	3 (60%)	4* (80%)	0 (0%)
slight		0 (0%)	0 (0%)	1 (20%)	5** (100%)
Score Expanded Totals		0 (0%)	3 (60%)	5** (100%)	5** (100%)
Wagner grade 1		5 (100%)	0** (0%)	0** (0%)	0** (0%)
Wagner grade 3		0 (0%)	5** (100%)	4* (80%)	0 (0%)
Wagner grade 4		0 (0%)	0 (0%)	1 (20%)	5** (100%)
EPS grade 0		5 (100%)	0** (0%)	0** (0%)	0** (0%)

Significance of difference in a pairwise Fisher's test between control and treatment groups: *P<0.05,**P<0.01,***P<0.001
Figures in brackets represent the number of animals from which this tissue was examined microscopically

*** Continued

Table 13 (cont.) Histopathological findings

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Summary of Histopathological Findings, 15 weeks postexposure subgroup

LESIONS	TREATMENT	INCIDENCE OF LESIONS (PERCE) Males			
		Clean Air	~15 F/ml	~50 F/ml	~150 F/ml
LUNGS		(5)	(5)	(5)	(5)
EPS grade 1		0 (0%)	5** (100%)	4* (80%)	0 (0%)
EPS grade 2		0 (0%)	0 (0%)	1 (20%)	5** (100%)
Aspiration pneumonia slight		0 (0%)	0 (0%)	1 (20%)	0 (0%)
Score Expanded Totals		0 (0%)	0 (0%)	1 (20%)	0 (0%)
Alveolar inflammatory cell infiltration very slight		0 (0%)	0 (0%)	1 (20%)	0 (0%)
Score Expanded Totals		0 (0%)	0 (0%)	1 (20%)	0 (0%)
Osseous metaplasia slight		0 (0%)	0 (0%)	1 (20%)	0 (0%)
Score Expanded Totals		0 (0%)	0 (0%)	1 (20%)	0 (0%)

Significance of difference in a pairwise Fisher's test between control and treatment groups: *P<0.05,**P<0.01,***P<0.001
Figures in brackets represent the number of animals from which this tissue was examined microscopically

*** Listing Complete ***

Table 14 Results of fibrosis scoring

Group	Lung fibrosis at postexposure date		Lung fibrosis at postexposure date		Lung fibrosis at postexposure date	
	1 Week		8 Week		15 Week	
	EPS grade +		EPS grade +		EPS grade +	
	MEAN	SD	MEAN	SD	MEAN	SD
Control	0.00	0.00	0.00	0.00	0.00	0.00
Carbon fiber low	***1.00	0.00	***1.00	0.00	***1.00	0.00
Carbon fiber med	***1.40	0.55	***1.20	0.45	***1.20	0.45
Carbon fiber high	***2.00	0.00	***2.00	0.00	***2.00	0.00

Group	Lung fibrosis at postexposure date				Lung fibrosis at postexposure date				Lung fibrosis at postexposure date			
	1 Week				8 Week				15 Week			
	Wagner grade +		Wagner grade 4 % of total parenchyma		Wagner grade +		Wagner grade 4 % of total parenchyma		Wagner grade +		Wagner grade 4 % of total parenchyma	
	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD	MEAN	SD
Control	1.00	0.00	ND		1.00	0.00	ND		1.00	0.00	ND	
Carbon fiber low	***3.00	0.00	ND		***3.00	0.00	ND		***3.00	0.00	ND	
Carbon fiber med	***3.40	0.55	0.74	0.09	***3.20	0.45	0.68	.	***3.20	0.45	0.93	.
Carbon fiber high	***4.00	0.00	1.61	0.23	***4.00	0.00	0.89	0.18	***4.00	0.00	1.08	0.13

Statistics: Anova + Dunnett's tests. (Two-Sided) * P<=5% ; ** P<=1% ; *** P<=0.1%
SD: Standard deviation
+ EPS grade 2 and Wagner grade 4 correspond to evidence of early interstitial fibrosis
ND Not detected (no animals with Wagner grade 4)

9.6.2 Time Course of Histopathological Findings

The time course of the main histopathological findings is given in **Table 15**.

Table 15 Summary of histopathological findings

Group	No. of animals (out of 5) <with indicated lesion				No. of animals (out of 5) <with indicated lesion				No. of animals (out of 5) <with indicated lesion			
	Postexposure Interval				Postexposure Interval				Postexposure Interval			
	1 weeks				8 weeks				15 weeks			
	Score				Score				Score			
	very slight	slight	mode-rate	total	very slight	slight	mode-rate	total	very slight	slight	mode-rate	total
Accumulation of fiber/particle-laden macrophages												
Control				0				0				0
Carbon fiber low	**5			**5	*4	1		**5	**5			**5
Carbon fiber med		**5		**5		**5		**5		**5		**5
Carbon fiber high		1	**4	**5		2	3	**5		1	**4	**5
Bronchiolo-alveolar hyperplasia												
Control				0				0				0
Carbon fiber low	*4	1		**5	3	2		**5	3	1		*4
Carbon fiber med		**5		**5		**5		**5	1	*4		**5
Carbon fiber high		**5		**5		**5		**5		**5		**5
Microgranulomas												
Control				0				0				0
Carbon fiber low	**5			**5	*4	1		**5	**5			**5
Carbon fiber med		**5		**5		**5		**5		**5		**5
Carbon fiber high		3	2	**5		3	2	**5		3	2	**5
Interstitial fibrosis												
Control				0				0				0
Carbon fiber low	**5			**5	**5			**5	3			3
Carbon fiber med	3	2		**5	*4	1		**5	*4	1		**5
Carbon fiber high		**5		**5		**5		**5		**5		**5

Significance of difference in a pairwise Fisher's test compared to control group : *P<0. 05, **P<0. 01, ***P<0 .001

9.6.3 Cell Proliferation Test

The results of the BrdU proliferation test are summarized in the **Table 16**.

Table 16 Proliferation index of lung tissue cells

Group	Unit length labelling index [%] of terminal bronchiolar epithelium at postexposure [positive cells per mm]								
	1 Week			8 Weeks			15 Weeks		
	Mean	SD	N	Mean	SD	N	Mean	SD	N
Control	2.14	1.47	5	3.29	2.00	5	2.30	1.25	5
Carbon fiber low	***9.77	1.88	5	6.73	3.09	5	*10.60	8.52	5
Carbon fiber med	***13.80	3.51	5	**12.70	4.14	5	**14.55	2.77	5
Carbon fiber high	***38.88	2.64	5	***15.11	3.92	5	***21.32	2.76	5
Group	Labelling index of lung parenchymal cells at postexposure [% positive cells]								
	1 Week			8 Weeks			15 Weeks		
	Mean	SD	N	Mean	SD	N	Mean	SD	N
Control	2.62	0.94	5	2.99	0.95	5	4.98	1.46	5
Carbon fiber low	3.31	0.46	5	4.03	1.03	5	4.86	1.16	5
Carbon fiber med	**4.25	0.43	5	*4.26	0.27	5	6.19	1.11	5
Carbon fiber high	***6.27	0.95	5	***5.39	0.50	5	***9.41	0.61	5
Group	Unit length labelling index [%] of pleural cells at postexposure [positive cells per cm]								
	1 Week			8 Weeks			15 Weeks		
	Mean	SD	N	Mean	SD	N	Mean	SD	N
Control	6.84	3.59	5	1.77	1.88	5	1.98	0.72	5
Carbon fiber low	7.63	3.98	5	3.76	0.81	5	2.95	1.56	5
Carbon fiber med	10.49	2.55	5	10.83	15.57	5	6.43	5.95	5
Carbon fiber high	*33.06	26.27	5	3.73	2.91	5	3.69	2.34	5

Statistics: Anova + Dunnett's tests. (Two-Sided) * P<=5% ; ** P<=1% ; *** P<=0.1% [a]
SD: Standard deviation N: Number of animals

10 STUDY PLAN ALTERATIONS

In one study plan amendment it was documented that for some filter samples with low content of fibers with length < 5 μm less than 100 fibers of that category were analyzed. Another amendment describes that the clinical chemistry was taken over by Dr. Hansen at the end of 2002. Additionally, in one amendment it was decided to change the last sacrifice date to February 26, 2003, to use the reserve animals (no. 32 of group 1, no. 31 and 32 of groups 2 to 4) for BAL at the last sacrifice date and to weigh and preserve the LALN at the last sacrifice date for optional fiber retention analysis.

In the 4th amendment it is documented that the exact sacrifice dates were 7.57 (BAL) and 7.71 weeks (Retention/Patho) which is rounded to 8 weeks instead of 7 in the study plan. For the last sacrifice date the exact dates were 14.71 (Retention/Patho) and 14.86 weeks (BAL) which has to be rounded to 15 weeks instead of 14 weeks in the study plan. Additionally a spelling mistake was corrected, the Tween concentration for resuspending of carbon fibers in lung tissue samples was 0.05 % instead of 0.5% in the study plan.

In the 5th amendment it is documented that the optional fiber analysis in the lungs for sacrifice dates 8 and 15 weeks postexposure and in the LALN at 15 weeks postexposure were performed on request of the sponsor.

In a deviation to the study plan it was documented that an additional signed report was drawn up for stage 1 of the study (containing results of exposure period and the first sacrifice date 1 week postexposure).

11 DISCUSSION

General

The aim of the study was to investigate the intrinsic toxicity of carbon fibers with a mean diameter of about 1 μm in rats after subchronic inhalation according to the protocol ECB/TM 16 (97) rev. 1.

The objectives of this study were:

- to investigate the effects of fibers on their inflammatory potential, the proliferation of alveolar epithelium, and the persistence of these effects during a recovery period of 3 months.

Rats were exposed 6 hours per day, 5 days per week for 3 months. The target dose was an exposure to 15, 50 and 150 fibers/ml at a fiber length > 20 µm. As **Table 3** shows these concentrations were reached.

The mean gravimetric concentrations in the exposure chambers were 2.1, 6.7 and 20.6 mg/m^3 for the three dose groups. The diameter of the fibers in the aerosol (see **Table 5**) was close to the value of 1.0 µm which is corresponding to the preferred 0.8 μm (fibers with density of 2.4) for the long fiber fraction (length > 20 µm) in the EC Guideline ECB/TM 16 Rev. 1. The mean geometric diameter of fibers with length > 20 µm were 1.14, 1.19, and 1.20 µm for low, medium and high dose, respectively. The corresponding values for the WHO fibers were 1.00, 1.04, and 1.03 μm.

After exposure of rats to the test fibers no biological effects were observed except that in the lungs.

Biological effects measured included the inflammatory and proliferative potential and histopathology lesions.

Body Weights

Body weight development during the exposure and posttreatment period did not show any evident differences between fiber treatment groups and the control group.

Wet Weights of Lungs

In the medium and high dose groups a statistically significant increase of the lung weight was observed up to 3 months postexposure (see **Table 6**). In the low dose group a significant increase was seen only at the first sacrifice date 1 week postexposure.

Retention of Test Fibers in the Lungs

After 3 months of exposure the lung retention of WHO fibers in fibers per lung was about 13 x 10^6, 62 x 10^6 and 145 x 10^6 for low, medium, and high dose, respectively (see **Table 7**). The corresponding values for long fibers (length > 20 µm) were about 3.3 x 10^6, 10.8 x 10^6 and 31.5 x 10^6 for the three groups. The corresponding values for the retained mass of fibers (estimation from the number and size of fibers in the lung ash) in the lungs in mg/lung were 0.27, 1.0, and 2.8 for low,

medium, and high dose, respectively. The estimated particle mass was less than 20% of the corresponding fiber mass in the lungs for all fiber groups.

After 3 months of recovery the WHO fiber concentration in the lungs decreased to 55%, 50% and 67% for the low, medium and high dose group, respectively. For the long fiber fraction with length > 20μm the corresponding data were 77%, 89% and 93% for the three dose groups. The elimination of fibers is slower in the high dose group compared to the low and medium dose group. Additionally the elimination of the long fiber fraction is much slower than for the WHO fiber fraction, which is different to the clearance behavior of man-made vitreous fibers. For most MMVFs a faster clearance was detected for the long fiber fraction compared to the WHO fiber fraction.

BAL

The effects on biochemical parameters (LDH and protein) in the BAL fluid were statistically significant at the end of the 3 months exposure and at 8 and 15 weeks postexposure for the high dose groups (**Table 11**). For the medium dose group the effect on protein was statistically significant and the effect on LDH was not significant for all sacrifice dates. For the low dose group only the protein was increased significantly only at end of exposure. The effect on ß-glucuronidase was significant only for the high dose group at end of 3 months exposure but not for later sacrifice dates. For the low and medium dose group the small increase of ß-glucuronidase was not significant for all sacrifice dates.

For medium and high dose fiber groups a statistically significant increase of neutrophil PMN and a reduction of the percentage of macrophages in the BALF was observed for all sacrifice dates, but the effect was less for 8 weeks and 15 weeks postexposure (**Table 12**). There was also a significant increase in lymphocytes in the mid and high dose groups up to 3 months postexposure. No significant effect was seen in the low dose group.

Histopathology

At the termination of the 3-month exposure period, very slight to moderate morphological changes were diagnosed (**Table 15**). These changes included alveolar macrophage aggregation and/or microgranulomas at the bronchiolo-alveolar junction in the few rats affected. At the end of exposure evidence of early interstitial fibrosis was detected for 2 rats of the medium dose group and for all 5 rats of the high dose group (**Table 15**). At 8 and 15 weeks postexposure this effect decreased to one rat of the medium dose group, but was retained at 5 rats for the high dose group

The cell proliferation was enhanced at end of exposure in the high dose group for the bronchiolar epithelium, for alveolar parenchymal cells and for pleural cells, in the medium group for the bronchiolar epithelium and for alveolar parenchymal cells, and in the low dose goup only for the bronchiolar epithelium (**Table 16**).

For the alveolar parenchymal cells the increase of cell proliferation persisted significantly for the high

dose group up to 3 months, but for the medium dose group it was no longer significant at 3 months postexposure. For the cell proliferation of the bronchiolar epithelium the increase retained significant up to 3 months postexposure for all treatment groups. This effect corresponds to the histopathological finding of bronchio-alveolar hyperplasia, which is a cellular response to fiber deposition and retention at the bronchioalveolar epithelium. This is an protective effect to increase the mucus production for enhancement of clearance of fibers in that region.

Overall assessment

In a previous study at Fraunhofer ITEM (Bellmann et al, 2003) three other fiber types (E-glass microfibers, stonewool MMVF21 and the biosoluble Calcium-Magnesium-Silicate [CMS] fiber) were investigated in a 90 day inhalation study using the same protocol (ECB/TM 16(97) rev. 1).

The effects for different parameters are plotted versus the retained number of long fibers (length>20μm) in Figures in Appendix 10.

The results of comparison the effects of carbon fibers to that of E-glass, MMVF21 and CMS are summarized in **Table 17.**

For the cell proliferation results a statistical analysis was done by comparison of carbon fiber induced effects to the dose groups of E-glass microfiber, MMVF21 and CMS fiber with comparable lung burden of test fibers (see App. 11). Only for the cell proliferation of the bronchiolar epithelium the increase induced by carbon fibers was significantly higher at 8 and 15 weeks postexposure comparing medium dose carbon fibers to E-glass medium dose and at 15 weeks postexposure when comparing low dose carbon fibers to MMVF21 medium dose.

Table 17 Effects of Carbonfibers in comparison to E-glass, MMVF21 and CMS (for the same lung retention of long fibers)

Parameter	E-glass			MMVF21			CMS		
	Postexposure interval [weeks]			Postexposure interval [weeks]			Postexposure interval [weeks]		
	1	8	15	1	8	15	1	8	15
LDH	>	≈	>	<	≈	>	<	≈	<
ß-Glucuron.	≈	<	>	<	≈	<	≈	≈	<
Total Protein	≈	≈	>	<	≈	≈	<	≈	<
PMN	<	<	<	<	<	<	<	<	<
Bronch-alv. Hyperplasia	>	>	≈	<	<	<	<	<	<
Mycrogranulomas	≈	≈	≈	<	≈	≈	<	<	<
Fibrosis EPS grade	≈	≈	<	<	<	<	<	<	<
Proliferation parenchymal cells	≈	>	*	<	<	*	<	≈	*
Proliferation Term. Bronchioles	<	>	>	<	>	>	<	>	>

> Effect of carbon fibers on this parameter is higher compared to other fibers at the same lung retention of long fibers at end of exposure

≈ Effect of carbon fibers on this parameter is comparable to other fibers at the same lung retention of long fibers at end of exposure

< Effect of carbon fibers on this parameter is lower compared to other fibers at the same lung retention of long fibers at end of exposure

* Significant differences between control group values

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Appendix 1 Body Weights

Control

Animal	Bodyweights [g] on Day										
No.	-10	-3	4	11	18	25	32	39	46	53	60
1001	188.6	219.6	244.2	268.9	294.0	313.9	325.2	340.7	341.3	351.7	357.7
1002	183.1	220.0	247.4	280.1	296.1	318.7	334.6	357.3	356.4	367.9	368.3
1003	209.6	246.3	267.1	288.3	312.8	335.2	350.9	361.3	362.7	373.9	379.9
1004	160.8	193.2	214.6	234.9	251.6	261.2	276.9	286.4	293.8	303.1	307.7
1005	196.9	227.7	254.8	278.0	295.5	311.6	316.7	324.8	326.8	338.3	339.4
1006	189.7	229.2	254.5	281.0	308.3	330.8	342.4	352.1	356.2	372.0	376.9
1007	209.6	251.4	271.0	297.7	323.8	344.5	357.4	369.4	378.2	385.1	393.2
1008	180.9	207.4	230.3	252.8	265.3	281.6	284.9	295.3	302.1	312.0	317.5
1009	190.1	228.8	264.9	287.9	307.2	325.6	337.2	351.2	358.0	369.3	373.3
1010	165.6	195.5	219.7	237.0	257.4	275.9	295.6	309.8	321.9	331.1	334.5
1011	167.5	202.7	228.9	253.0	274.4	293.2	309.4	319.7	325.5	337.5	344.0
1012	168.1	204.9	224.5	246.8	264.0	279.1	291.8	297.8	300.4	309.3	313.2
1013	199.8	232.3	255.1	275.5	297.7	313.9	331.7	343.1	349.2	359.1	364.7
1014	182.4	214.1	255.1	262.9	325.6	299.0	317.0	330.8	340.5	356.8	359.7
1015	205.7	243.9	272.7	297.2	316.5	332.8	353.7	365.8	380.0	390.8	395.3
1016	184.9	217.2	239.3	252.5	294.7	287.5	302.1	315.4	323.2	331.7	332.9
1017	184.4	221.3	247.3	272.6	307.9	312.3	328.1	346.4	355.9	370.0	378.8
1018	206.3	244.2	268.2	287.4	308.2	324.5	340.7	352.4	359.5	369.5	377.9
1019	179.3	211.8	233.3	255.3	272.3	291.9	302.2	312.3	319.5	334.5	339.1
1020	184.8	219.6	241.2	258.7	281.3	301.8	311.0	321.8	330.5	343.2	342.0
1021	226.1	263.2	295.1	319.6	345.6	365.7	380.1	396.7	404.3	426.0	427.6
1022	190.6	225.8	258.5	283.0	305.9	328.2	345.3	363.6	370.6	385.6	392.1
1023	173.1	205.1	225.2	245.9	263.5	281.6	296.3	308.5	313.2	327.8	330.1
1024	211.0	249.3	279.1	306.6	329.6	336.5	356.5	371.8	373.3	392.0	401.2
1025	184.4	224.3	256.8	279.2	303.8	324.9	338.0	350.8	360.5	370.5	380.4
1026	201.1	242.6	269.7	290.0	308.3	329.7	342.7	354.8	365.6	374.9	381.2
1027	215.8	251.5	288.4	318.8	341.5	361.7	377.2	390.3	399.0	411.1	416.0
1028	175.9	217.5	245.5	259.5	280.9	293.8	303.5	314.2	318.0	328.3	334.3
1029	189.9	226.1	256.9	283.4	298.0	314.6	317.1	330.6	334.7	342.0	348.8
1030	213.4	252.9	284.9	306.1	325.1	346.4	357.8	377.7	392.1	403.1	407.3
1031	174.9	219.8	248.8	270.4	293.0	303.5	317.1	320.9	333.1	345.0	350.6
1032	169.2	205.5	232.0	249.0	272.2	291.2	300.3	312.0	311.3	333.8	335.0
MEAN	189.5	225.5	252.3	274.4	297.6	312.9	326.3	338.9	345.5	357.7	362.5
SD	16.6	18.1	20.5	22.6	24.2	25.3	26.4	28.2	29.2	30.0	30.7
N	32	32	32	32	32	32	32	32	32	32	32

Appendix 1 (cont.) Body Weights

Carbon fiber low

Animal	Bodyweights [g] on Day										
No.	-10	-3	4	11	18	25	32	39	46	53	60
2001	187.5	224.7	252.0	279.0	298.6	318.4	336.3	348.5	358.0	367.3	369.4
2002	179.6	218.8	247.5	267.3	285.0	308.5	324.4	338.0	344.0	360.8	365.7
2003	173.1	188.1	214.1	230.3	244.2	261.5	274.4	278.1	276.1	286.8	290.9
2004	196.5	230.8	259.2	287.5	310.6	331.5	353.6	369.0	373.5	392.5	401.3
2005	172.3	209.9	235.9	254.0	271.5	291.7	307.0	317.8	320.8	335.6	335.9
2006	171.7	205.8	233.3	255.2	275.5	296.9	308.6	324.2	325.3	339.0	344.8
2007	193.2	227.4	255.9	278.6	295.9	310.4	321.6	336.0	341.9	350.0	359.0
2008	196.1	229.7	257.4	282.2	301.9	323.3	335.0	346.2	354.5	363.3	374.3
2009	193.0	231.1	260.7	289.5	307.8	326.8	338.2	350.1	350.9	365.2	366.5
2010	194.1	231.9	247.3	259.3	271.6	287.7	302.9	310.9	311.2	325.8	319.8
2011	180.2	217.8	239.5	262.8	278.0	294.1	303.9	318.6	329.2	336.9	344.6
2012	180.0	211.5	236.0	258.0	272.8	292.2	307.8	319.5	325.5	334.9	342.8
2013	190.1	225.1	251.4	273.9	293.2	309.9	321.3	334.2	339.3	352.8	359.0
2014	186.3	222.7	245.3	271.3	292.2	313.9	329.7	337.5	344.5	343.0	358.0
2015	188.3	215.3	242.9	259.9	276.7	290.0	301.6	315.9	321.1	330.1	334.3
2016	165.4	198.7	223.9	246.4	263.2	282.2	299.0	318.1	324.5	334.2	342.4
2017	182.0	228.0	254.4	276.0	296.2	312.0	325.1	340.7	347.1	358.9	364.6
2018	161.8	198.8	231.1	258.0	281.4	305.0	321.1	332.9	346.0	366.5	373.9
2019	163.5	194.3	221.4	242.3	257.1	269.5	276.8	284.2	289.3	300.4	300.3
2020	194.3	228.5	253.6	282.8	298.7	317.6	333.5	345.5	357.3	373.8	380.0
2021	167.2	203.1	235.6	258.2	276.2	299.0	313.0	327.4	334.9	349.3	356.0
2022	172.0	205.5	237.8	260.4	277.8	296.2	307.0	316.7	325.8	336.6	343.1
2023	194.7	234.8	261.4	282.5	301.4	326.4	345.4	358.4	366.4	385.4	388.5
2024	192.4	224.8	248.5	275.4	286.9	306.0	322.7	336.5	345.5	358.6	359.6
2025	200.8	234.1	268.6	296.1	315.6	337.1	350.3	363.3	371.3	389.8	387.3
2026	177.4	220.9	248.0	274.6	290.6	312.0	327.4	339.0	342.9	356.2	356.6
2027	168.0	203.4	235.7	254.8	274.4	288.9	304.0	320.4	330.2	345.3	344.6
2028	187.0	219.5	250.3	267.5	292.4	309.6	326.4	344.5	350.3	363.5	363.3
2029	187.1	224.9	253.9	281.8	300.4	316.6	338.0	348.1	363.7	376.0	388.7
2030	184.9	220.6	246.9	274.4	294.9	309.9	323.7	334.5	344.3	357.8	365.6
2031	174.8	203.7	231.8	249.9	267.4	282.8	298.8	309.5	315.2	330.3	328.4
2032	179.4	209.7	235.1	255.4	272.7	285.0	295.3	298.9	305.5	316.6	320.7
MEAN	182.3	217.0	244.3	267.0	285.1	303.5	317.9	330.1	336.8	349.5	354.1
SD	10.9	12.7	12.6	15.0	16.2	17.7	19.2	20.7	22.3	23.8	25.0
N	32	32	32	32	32	32	32	32	32	32	32

Appendix 1 (cont.) Body Weights

Carbon fiber medium

Animal	Bodyweights [g] on Day										
No.	-10	-3	4	11	18	25	32	39	46	53	60
3001	207.9	242.9	269.3	295.7	315.6	334.5	354.7	368.2	374.1	392.6	393.5
3002	188.4	220.5	248.9	277.1	294.3	318.0	330.6	347.1	356.6	366.9	367.0
3003	194.2	234.8	259.0	284.9	306.4	325.3	340.3	354.6	361.4	373.7	385.2
3004	179.5	214.0	234.4	262.5	281.8	295.0	311.8	322.1	327.2	335.3	331.4
3005	202.0	236.7	258.6	282.0	298.0	316.7	326.1	337.9	341.6	355.8	353.5
3006	172.9	208.2	234.9	262.7	279.7	302.2	314.9	330.4	335.4	350.5	353.4
3007	197.2	226.0	251.4	278.3	295.2	315.8	332.4	341.1	347.7	360.0	357.7
3008	193.4	224.2	252.8	280.4	297.7	319.4	331.5	345.9	354.3	368.5	366.8
3009	189.1	233.0	264.5	295.7	316.8	344.5	360.3	372.7	382.0	396.2	399.5
3010	185.0	224.0	247.2	272.5	288.6	304.3	315.8	326.3	329.3	336.5	345.5
3011	192.1	223.2	252.3	281.0	300.1	312.6	327.8	332.9	333.0	356.3	360.0
3012	179.0	218.5	236.7	264.7	285.1	307.4	319.0	330.0	343.2	353.7	353.3
3013	184.1	224.3	256.2	283.5	299.7	314.5	331.7	342.3	352.5	359.5	372.4
3014	167.3	197.4	216.2	239.8	255.2	269.4	281.9	294.6	296.7	310.2	318.7
3015	215.3	248.8	269.2	293.3	304.4	325.6	338.6	348.2	355.8	366.2	368.1
3016	164.4	198.6	223.8	249.7	261.6	282.2	295.4	308.9	314.4	326.3	332.3
3017	172.4	200.3	221.9	247.0	261.0	279.2	286.0	298.5	303.2	318.8	322.4
3018	182.8	220.7	245.4	273.3	289.2	311.4	323.1	338.4	345.8	359.6	356.4
3019	167.7	200.9	233.2	261.3	278.0	298.1	313.6	324.7	331.0	341.5	347.4
3020	169.7	200.0	222.0	245.9	257.8	273.1	286.6	301.9	315.7	327.4	333.5
3021	178.0	218.8	248.7	274.9	291.7	309.8	323.5	340.3	349.6	363.7	370.0
3022	176.8	208.1	232.5	227.0	247.3	268.6	286.4	297.5	297.7	304.3	306.5
3023	184.7	217.1	236.9	259.2	272.5	286.3	301.9	313.2	326.4	338.3	342.6
3024	189.1	223.1	253.1	277.5	290.8	307.8	316.9	330.6	337.7	352.5	352.9
3025	191.8	224.6	251.9	277.0	295.5	308.1	324.4	328.3	338.1	355.9	356.2
3026	190.0	231.8	262.2	292.8	309.8	324.8	347.0	359.7	369.7	384.9	388.8
3027	170.0	200.6	225.6	241.1	253.2	270.0	285.9	296.9	304.6	312.3	313.9
3028	194.0	235.4	264.6	294.9	314.2	334.0	345.7	357.0	364.1	376.9	379.3
3029	226.0	260.5	291.7	323.3	337.5	359.7	378.9	391.1	400.8	413.9	422.6
3030	194.9	227.0	252.7	282.0	293.4	310.7	324.8	335.4	340.3	354.0	355.3
3031	177.4	215.7	240.1	268.2	286.9	307.6	317.0	325.0	333.0	344.2	339.6
3032	181.0	220.5	241.2	272.6	281.8	300.3	311.7	322.4	325.8	343.8	342.6
MEAN	186.2	221.3	246.8	272.6	288.8	307.4	321.4	333.3	340.3	353.1	355.9
SD	14.0	15.0	16.6	19.9	20.7	21.8	22.9	23.0	24.1	25.1	25.6
N	32	32	32	32	32	32	32	32	32	32	32

Appendix 1 (cont.) Body Weights

Carbon fiber high

Animal	Bodyweights [g] on Day										
No.	-10	-3	4	11	18	25	32	39	46	53	60
4001	176.3	207.0	233.3	257.5	277.6	295.0	312.1	329.3	333.2	342.0	353.3
4002	201.5	239.2	269.2	291.6	316.5	328.6	349.2	364.9	371.9	380.2	387.1
4003	168.9	199.5	220.6	237.8	254.5	272.9	280.9	290.3	299.0	309.0	316.3
4004	193.0	230.7	258.8	290.1	313.3	338.4	349.3	361.6	368.0	386.6	392.9
4005	198.7	233.7	262.3	290.0	310.1	332.2	350.5	361.1	370.1	384.0	385.6
4006	193.9	221.6	240.9	261.2	275.0	289.1	309.9	323.8	327.7	340.8	351.4
4007	199.4	237.4	258.8	279.3	297.2	313.8	325.9	329.4	335.6	348.4	353.3
4008	213.3	245.6	269.9	292.5	314.0	330.6	336.5	343.9	355.1	371.6	377.3
4009	206.6	243.0	259.1	279.9	297.9	314.0	331.3	345.6	348.7	358.9	365.6
4010	155.3	191.4	218.5	237.0	256.0	269.2	281.0	288.1	295.2	304.9	311.5
4011	170.8	208.1	233.0	254.3	274.8	292.8	306.3	316.9	328.0	336.6	340.9
4012	176.3	206.4	224.3	245.9	266.9	282.5	298.1	311.1	320.9	335.1	345.6
4013	159.6	236.3	260.2	282.1	300.0	318.9	331.1	343.4	350.3	365.1	373.7
4014	199.3	234.8	252.3	274.8	298.8	318.8	333.2	347.0	350.0	368.8	376.0
4015	188.5	224.4	245.9	275.4	297.8	319.8	339.8	353.0	365.2	374.5	385.9
4016	192.4	228.1	255.7	280.5	304.1	320.9	340.4	349.8	355.1	366.7	369.4
4017	189.7	225.9	245.3	266.9	284.6	291.8	303.8	315.5	316.3	329.0	331.0
4018	185.3	219.9	247.8	270.7	294.8	316.0	335.3	346.2	356.2	365.1	371.0
4019	169.3	202.4	224.4	244.9	263.9	275.9	289.5	304.9	316.9	331.7	338.3
4020	186.7	225.1	252.2	276.0	297.6	319.6	329.9	344.7	350.4	366.6	375.0
4021	165.6	198.1	214.0	229.9	247.6	259.0	270.5	281.0	288.0	299.2	303.8
4022	180.6	215.9	247.2	271.8	289.7	304.5	315.7	324.2	335.3	352.4	363.8
4023	169.1	200.8	218.1	230.7	238.6	248.7	252.0	267.1	276.2	280.6	285.1
4024	164.8	196.3	220.2	241.6	253.7	273.3	284.6	299.6	309.6	314.3	324.9
4025	178.8	217.3	248.2	266.9	288.0	301.9	318.7	327.2	338.6	350.4	350.7
4026	200.1	235.6	264.2	287.2	310.7	325.8	346.0	354.1	370.8	380.4	389.1
4027	190.8	227.2	247.9	269.0	285.1	300.0	310.0	324.9	331.9	345.4	343.0
4028	197.4	226.4	250.4	268.0	289.0	299.2	312.9	325.9	330.7	343.5	348.3
4029	177.0	209.2	237.0	253.9	274.6	288.8	304.1	311.9	321.8	334.7	340.7
4030	165.7	197.5	221.6	237.4	254.3	264.2	278.1	291.8	298.7	309.5	317.0
4031	197.1	235.9	267.8	292.1	317.5	337.6	346.9	359.6	369.4	383.2	392.2
4032	183.4	214.9	236.5	257.6	271.9	287.7	298.8	309.0	315.6	331.0	332.4
MEAN	184.2	219.9	243.9	265.5	*284.9	301.0	314.8	326.5	334.4	346.6	352.9
SD	14.9	15.6	16.9	19.4	22.1	24.3	26.1	26.0	26.2	27.6	28.1
N	32	32	32	32	32	32	32	32	32	32	32

Appendix 1 (cont.) Body Weights

Control

Animal	Bodyweights [g] on day											
No.	67	74	81	88	95	102	116	130	144	158	172	186
1001	361.4	369.7	356.0	366.4	371.9							
1002	372.9	388.8	395.2	405.4	400.4							
1003	382.6	389.5	399.4	411.3	419.0							
1004	311.0	313.5	319.7	321.0								
1005	338.9	343.9	350.3	357.9	358.8							
1006	384.3	389.7	399.1	403.7	405.1	418.4	429.1	448.7	463.8			
1007	401.3	407.1	409.6	418.4	427.0	431.6	445.6	470.8	474.8			
1008	322.6	327.6	335.5	342.7	345.6	353.6	364.7	375.3	392.9			
1009	381.6	386.3	394.2	400.9	400.7	407.1	420.8	441.4	450.0			
1010	337.2	341.1	344.8	353.6	354.0	361.0	367.2	385.3	394.9			
1011	351.6	359.9	372.3	383.5	384.9	394.2	400.3	425.9	441.9	452.7	442.6	455.8
1012	323.4	330.1	329.8	339.5	337.8	342.9	349.1	361.8	374.2	377.8	384.7	390.7
1013	371.1	381.1	388.2	396.3	396.5	409.1	420.2	434.3	449.5	457.5	465.2	466.6
1014	370.1	376.0	385.5	387.4	395.1	401.5	410.8	434.3	455.3	465.1	475.0	474.3
1015	370.5	385.7	400.6	414.1	417.3	429.4	446.4	467.1	483.2	489.0	501.7	509.2
1016	341.6	343.7	355.0	361.1	359.9							
1017	382.5	387.8	393.2	405.6	413.7							
1018	379.4	383.8	392.0	394.0	401.0							
1019	343.8	347.4	352.0	361.1	367.2							
1020	347.8	350.8	357.9	368.6	373.8							
1021	435.2	441.3	448.8	455.1	458.5	460.0	468.4	494.1	507.7			
1022	394.9	407.2	411.9	422.4	418.5	434.5	452.8	477.9	495.0			
1023	331.6	338.3	342.3	345.0	349.3	357.4	368.2	383.6	396.8			
1024	405.6	412.3	417.5	430.8	435.1	438.5	448.4	462.6	471.4			
1025	386.5	391.9	404.0	408.7	412.5	423.3	427.6	445.6	454.5			
1026	386.0	392.2	400.5	410.5	414.2	417.7	423.4	440.5	460.3	466.6	478.9	471.2
1027	421.3	431.7	437.5	444.9	455.3	465.8	478.4	499.5	512.3	518.2	517.5	526.3
1028	342.8	343.4	352.4	353.5	363.0	373.3	386.6	403.6	411.4	417.5	429.1	430.8
1029	351.6	357.8	366.3	370.7	371.3	374.3	377.5	403.5	421.0	427.6	435.2	439.5
1030	405.4	417.2	428.8	431.2	434.5	445.5	458.0	478.3	493.4	500.3	511.5	511.2
1031	349.1	349.5	362.0	369.4	356.1							
1032	338.7	343.9	349.8	360.9	364.6	368.5	378.2	399.7	419.7	413.9	430.6	439.1
MEAN	366.4	372.8	379.8	387.4	392.3	405.1	415.3	434.9	448.8	453.3	461.1	465.0
SD	30.1	31.8	32.7	33.4	32.9	36.4	38.0	40.1	39.9	41.5	40.9	40.1
N	32	32	32	32	31	21	21	21	21	11	11	11

Appendix 1 (cont.) Body Weights

Carbon fiber low

Animal	Bodyweights [g] on day											
No.	67	74	81	88	95	102	116	130	144	158	172	186
2001	369.6	374.1	386.2	393.9	395.9							
2002	369.0	372.2	378.5	390.1	391.4							
2003	291.8	301.9	311.7	316.3	321.9							
2004	406.9	414.8	420.6	430.4	432.3							
2005	339.7	347.1	353.4	361.6	362.6							
2006	351.3	359.4	369.7	377.4	379.4	389.5	396.3	413.9	426.4			
2007	362.8	366.6	371.7	380.0	382.8	386.9	391.2	411.4	412.5			
2008	382.2	387.1	396.3	403.3	403.9	414.7	427.4	448.1	456.9			
2009	376.1	387.0	394.4	400.7	400.3	410.0	424.9	443.1	463.8			
2010	330.9	334.6	346.7	346.8	343.2	349.6	361.4	376.4	393.4			
2011	349.6	353.6	353.8	359.5	364.1	372.6	387.2	405.7	416.2	421.6	422.6	431.8
2012	342.8	346.3	355.0	368.2	370.2	368.8	374.9	393.2	407.2	416.5	423.1	426.0
2013	368.6	376.5	385.0	388.7	391.0	398.1	411.2	426.4	435.6	440.5	451.7	454.4
2014	367.1	375.5	380.8	384.5	381.4	386.9	394.5	407.7	422.4	422.6	432.0	444.7
2015	338.7	344.2	351.6	356.2	361.2	371.1	377.8	381.7	405.4	412.7	416.5	420.1
2016	342.0	345.8	350.4	355.1	356.9							
2017	367.6	374.5	379.8	385.6	389.2							
2018	382.3	386.3	398.0	404.4	411.0							
2019	303.3	314.3	318.4	324.8	327.8							
2020	386.9	396.7	405.1	415.9	416.8							
2021	358.6	363.6	378.2	384.6	388.3	393.1	410.3	429.3	453.8			
2022	348.6	352.8	367.3	371.6	371.1	375.1	384.2	399.2	414.3			
2023	394.0	400.6	406.4	422.5	421.8	429.3	447.8	464.1	487.7			
2024	366.8	372.2	381.5	390.0	392.4	400.6	416.0	436.6	449.6			
2025	393.4	401.3	411.8	412.5	413.6	424.3	429.5	448.0	462.9			
2026	367.9	370.2	373.9	377.8	384.8	397.0	402.2	417.5	428.2	436.8	441.7	455.6
2027	347.1	357.5	365.4	373.7	374.1	387.6	396.8	417.3	440.6	448.0	455.7	463.0
2028	368.3	376.1	378.2	392.3	399.5	403.3	407.6	423.3	438.1	435.1	449.5	451.4
2029	395.2	402.3	409.3	423.4	426.4	436.2	448.8	461.8	484.0	486.8	496.8	503.7
2030	371.8	374.6	381.0	386.2	385.0	396.9	411.1	426.3	442.0	447.4	459.1	460.5
2031	334.8	339.5	352.7	363.1	368.4	377.4	396.3	415.8	434.6	446.6	457.7	468.7
2032	319.3	325.3	333.5	341.1	341.3	338.5	348.3	368.4	389.2	393.3	402.7	412.8
MEAN	359.2	365.5	373.3	380.7	382.8	391.3	402.1	418.9	434.8	434.0	442.4	449.4
SD	26.2	26.1	26.1	27.3	27.1	24.0	25.2	25.7	26.7	23.5	25.1	24.8
N	32	32	32	32	32	22	22	22	22	12	12	12

Appendix 1 (cont.) Body Weights

Carbon fiber medium

Animal	Bodyweights [g] on day											
No.	67	74	81	88	95	102	116	130	144	158	172	186
3001	402.5	404.9	413.4	423.1	419.9							
3002	372.6	374.6	386.9	394.4	397.1							
3003	389.9	391.4	407.6	413.9	414.1							
3004	331.6	343.4	357.7	370.6	373.9							
3005	359.2	364.0	371.2	374.7	379.4							
3006	357.5	368.7	375.3	380.9	383.2	390.1	402.8	415.7	423.7			
3007	362.4	371.4	383.9	387.7	393.5	399.9	405.6	422.4	423.6			
3008	375.2	383.7	393.7	403.9	413.2	418.3	418.7	439.8	447.2			
3009	405.3	410.0	419.0	423.7	423.5	429.7	442.7	446.9	462.3			
3010	347.9	337.7	347.2	352.6	357.3	357.2	360.5	372.8	382.2			
3011	366.0	372.2	381.9	389.9	398.1	401.1	407.1	427.4	438.9	453.4	459.0	469.5
3012	354.7	361.0	368.8	375.2	372.6	383.1	387.5	402.5	408.3	418.8	427.0	435.2
3013	371.0	376.8	387.3	394.4	399.0	408.3	416.2	438.9	459.2	466.3	481.0	490.9
3014	315.7	325.8	326.6	331.0	329.9	333.1	344.3	355.1	376.2	383.6	388.4	397.3
3015	374.8	378.0	387.4	396.5	400.9	405.5	420.3	436.8	449.5	459.5	474.9	484.2
3016	336.2	339.8	346.7	355.6	363.9							
3017	325.2	329.7	344.1	352.6	351.2							
3018	356.5	360.9	382.1	387.2	393.9							
3019	347.4	356.7	364.5	367.0	374.4							
3020	336.4	344.1	349.1	364.2	361.8							
3021	375.6	377.9	380.5	393.3	392.4	402.0	411.3	429.4	450.6			
3022	306.7	312.9	315.1	320.8	323.4	329.3	340.3	356.7	365.3			
3023	344.5	349.6	352.9	366.0	369.3	375.7	384.9	402.2	418.9			
3024	357.4	360.7	373.0	387.6	395.4	399.2	418.8	436.9	458.7			
3025	357.2	364.3	360.6	372.6	373.1	383.7	392.3	400.9	415.2			
3026	392.7	396.9	402.9	410.6	410.4	425.3	440.7	463.5	478.2	487.0	492.4	499.1
3027	321.5	324.9	335.4	342.1	345.5	350.5	371.0	389.4	401.3	414.5	426.2	432.2
3028	382.0	388.6	397.3	402.5	403.9	411.5	425.9	440.1	448.9	456.1	464.6	473.5
3029	424.0	432.8	441.2	444.8	446.4	455.6	424.0	472.9	498.9	514.5	522.6	525.8
3030	360.1	372.5	385.2	387.8	383.0	392.0	410.0	430.8	448.8	462.4	474.9	478.3
3031	349.2	361.2	370.7	380.4	379.7	381.7	392.8	410.5	427.8	435.0	441.8	439.3
3032	350.8	359.0	364.2	372.1	374.4	385.0	398.9	416.9	435.1	447.3	457.5	466.0
MEAN	359.7	365.5	374.2	381.9	384.3	391.7	400.8	418.6	432.7	449.9	459.2	465.9
SD	26.4	26.2	27.4	27.0	26.9	30.3	27.6	30.7	32.8	34.3	35.1	34.9
N	32	32	32	32	32	22	22	22	22	12	12	12

Appendix 1 (cont.) Body Weights

Carbon fiber high

Animal	Bodyweights [g] on day											
No.	67	74	81	88	95	102	116	130	144	158	172	186
4001	361.8	364.2	369.7	374.5	375.0							
4002	391.1	391.7	390.3	392.7	393.7							
4003	313.4	318.2	318.8	326.3	326.5							
4004	400.6	408.5	417.6	430.9	431.2							
4005	384.8	386.2	395.8	395.9	398.1							
4006	353.9	357.6	368.2	371.5	376.6	394.1	414.0	432.0	448.6			
4007	360.4	364.8	361.0	367.0	368.1	377.8	388.7	406.6	418.2			
4008	377.8	383.7	387.2	391.3	389.5	400.8	415.6	433.9	448.7			
4009	366.3	369.9	377.5	386.6	384.3	388.1	403.2	423.1	427.0			
4010	313.1	323.9	328.3	334.0	328.9	334.9	343.9	357.4	376.0			
4011	342.1	343.9	346.4	338.7	343.0	356.6	371.6	395.0	408.1	410.1	418.0	429.7
4012	345.0	354.4	355.0	364.3	360.8	370.6	384.0	403.5	420.3	428.9	439.2	450.1
4013	373.7	380.8	384.9	388.1	393.7	402.2	414.8	438.7	451.5	461.7	469.8	473.7
4014	385.2	392.8	399.8	408.0	411.0	421.7	437.2	453.2	470.7	477.0	486.0	488.4
4015	391.2	396.7	407.9	417.2	419.7	423.8	437.1	454.3	471.5	474.7	492.9	503.4
4016	378.0	382.7	394.7	399.6	403.3							
4017	338.2	323.7	329.9	341.2	345.8							
4018	379.1	380.5	388.6	395.4	395.0							
4019	340.1	345.6	354.2	357.7	359.5							
4020	379.6	386.4	390.1	400.2	400.6							
4021	305.6	311.6	323.1	327.1	329.7	337.2	351.1	363.0	374.6			
4022	365.0	370.1	376.7	382.8	388.5	394.6	408.4	425.0	438.7			
4023	287.0	291.7	299.9	305.8	302.7	318.3	333.1	348.3	353.3			
4024	326.7	334.2	336.2	346.8	348.1	350.2	362.2	382.7	395.2			
4025	351.6	356.8	362.6	354.5	366.2	376.0	389.0	409.9	418.4			
4026	394.5	397.4	404.5	413.5	414.6	428.1	435.7	461.5	483.2	488.7	496.8	494.3
4027	353.8	355.7	361.9	364.2	355.0	364.4	378.8	399.6	418.4	427.2	437.4	445.2
4028	353.8	361.6	364.4	367.1	369.6	380.4	387.0	405.0	430.4	440.4	448.2	453.1
4029	341.8	347.2	352.3	358.1	358.4	363.5	375.1	388.4	402.4	412.4	419.7	430.1
4030	320.0	326.3	332.2	335.9	337.8	341.6	349.7	366.2	387.7	397.0	405.8	409.3
4031	392.4	391.1	391.4	398.2	401.7	401.9	413.4	430.4	439.5	452.2	458.1	471.4
4032	332.9	334.6	341.3	342.1	343.6	352.1	364.7	381.0	403.7	411.6	415.7	422.4
MEAN	356.3	360.5	366.0	371.2	*372.5	**376.3	*389.0	*407.2	*422.1	440.2	449.0	455.9
SD	29.1	29.1	29.4	30.6	31.2	30.5	30.9	32.5	33.4	30.4	31.8	30.4
N	32	32	32	32	32	22	22	22	22	12	12	12

Appendix 2a Terminal Body Weights and Lung Weights

Group	Sacrifice (weeks)	Animal no.	Lung wet weight [g]	Terminal Body weight [g]
Control	1	1001	1.244	367.5
		1002	1.357	399.0
		1003	1.403	418.5
		1005	1.310	359.4
		1016	1.275	358.1
		1017	1.465	410.7
		1018	1.276	400.9
		1019	1.243	365.7
		1020	1.287	367.8
		1031	1.279	354.8
	8	1006	1.587	461.5
		1007	1.489	475.5
		1008	1.415	389.6
		1009	1.538	446.8
		1010	1.266	394.3
		1021	1.515	507.7
		1022	1.376	494.7
		1023	1.132	396.6
		1024	1.227	471.0
		1025	1.397	453.7
	15	1011	1.433	447.2
		1012	1.306	388.0
		1013	1.433	460.9
		1014	1.503	460.4
		1015	1.436	495.9
		1026	1.657	459.8
		1027	1.628	532.4
		1028	1.216	436.6
		1029	1.595	442.0
		1030	1.570	508.2
		1032	1.299	439.4

Appendix 2a (cont.) Terminal Body Weights and Lung Weights

Group	Sacrifice (weeks)	Animal no.	Lung wet weight [g]	Terminal Body weight [g]
Carbon fiber low	1	2001	1.412	396.8
		2002	1.377	389.9
		2003	1.309	321.4
		2004	1.522	434.7
		2005	1.426	365.8
		2016	1.512	354.9
		2017	1.405	390.5
		2018	1.448	410.7
		2019	1.388	327.8
		2020	1.489	415.3
	8	2006	1.464	426.0
		2007	1.417	413.4
		2008	1.496	452.4
		2009	1.484	456.1
		2010	1.477	391.3
		2021	1.401	454.0
		2022	1.481	414.5
		2023	1.541	485.7
		2024	1.395	447.6
		2025	1.568	462.9
	15	2011	1.451	427.3
		2012	1.519	421.3
		2013	1.556	432.6
		2014	1.561	436.6
		2015	1.510	418.5
		2026	1.520	455.7
		2027	1.467	470.6
		2028	1.589	447.7
		2029	1.642	499.4
		2030	1.460	461.7
		2031	1.622	464.6
		2032	1.355	409.8

Appendix 2a (cont.) Terminal Body Weights and Lung Weights

Group	Sacrifice (weeks)	Animal no.	Lung wet weight [g]	Terminal Body weight [g]
Carbon fiber med	1	3001	1.647	419.0
		3002	1.539	393.4
		3003	1.661	406.7
		3004	1.536	370.2
		3005	1.530	381.7
		3016	1.576	362.5
		3017	1.583	349.5
		3018	1.719	392.7
		3019	1.647	374.7
		3020	1.622	360.1
	8	3006	1.630	423.3
		3007	1.625	418.5
		3008	1.637	442.1
		3009	1.701	460.0
		3010	1.550	379.5
		3021	1.567	450.5
		3022	1.402	364.8
		3023	1.466	419.6
		3024	1.657	458.8
		3025	1.502	415.8
	15	3011	1.641	453.8
		3012	1.675	422.7
		3013	1.789	465.6
		3014	1.431	389.1
		3015	1.781	484.5
		3026	1.730	506.9
		3027	1.447	434.5
		3028	1.648	472.9
		3029	2.068	528.1
		3030	1.651	478.5
		3031	1.551	436.3
		3032	1.734	469.7

Appendix 2a (cont.) Terminal Body Weights and Lung Weights

Group	Sacrifice (weeks)	Animal no.	Lung wet weight [g]	Terminal Body weight [g]
Carbon fiber high	1	4001	1.793	376.1
		4002	1.758	395.0
		4003	1.553	323.2
		4004	1.878	430.7
		4005	1.759	393.7
		4016	1.908	402.4
		4017	1.663	344.3
		4018	1.757	395.8
		4019	1.718	356.2
		4020	1.948	399.6
	8	4006	1.970	445.1
		4007	1.702	414.3
		4008	1.728	445.0
		4009	1.891	422.1
		4010	1.495	370.9
		4021	1.508	373.6
		4022	1.733	436.1
		4023	1.613	351.4
		4024	1.579	394.9
		4025	1.752	418.0
	15	4011	1.744	420.2
		4012	1.658	441.3
		4013	1.896	471.2
		4014	1.819	474.1
		4015	2.028	491.7
		4026	2.103	489.5
		4027	1.798	450.2
		4028	1.820	455.8
		4029	1.649	425.1
		4030	1.537	408.4
		4031	1.921	467.7
		4032	1.793	420.6

Appendix 2b Necropsy Observations

Organ	Finding	Incidence of finding											
		Sacrifice date (weeks postexposure)											
		1				8				15			
		Group				Group				Group			
		01	02	03	04	01	02	03	04	01	02	03	04
Lung	white areas		3				2			1	3		
	white and grey areas			10	3		3	10				12	7
	white and grey areas slightly bulging				7				7				5
	spongy consistency									1			
	grey glassy node												1
	red areas						1				1		
Kidney	cystic dilated							2					
Seminal vesicles	red areas								1				
	enlarged, solid consistency										1		

Appendix 3a Biochemical Parameters Measured in Bronchoalveolar Lavage (BAL)

Group	Sacrifice (weeks)	Animal No.	LDH [U/l]	ß-Glucoronidase [U/l]	Total Protein [mg/l]
Control	1	1016	33	0.1	85
		1017	27	0.2	92
		1018	47	0.3	105
		1019	41	0.3	104
		1020	43	0.3	109
	8	1021	31	0.3	112
		1022	34	0.2	115
		1023	31	0.2	91
		1024	36	0.3	98
		1025	38	0.3	108
	15	1026	47	0.3	119
		1027	27	0.2	89
		1028	38	0.4	125
		1029	33	0.1	106
		1030	19	0.2	91
		1032	33	0.2	89
Carbon fiber low	1	2016	50	0.2	143
		2017	57	0.4	141
		2018	59	0.2	144
		2019	63	0.3	171
		2020	106	0.3	161
	8	2021	41	0.1	119
		2022	51	0.2	139
		2023	59	0.4	193
		2024	39	0.3	120
		2025	41	0.3	136
	15	2026	37	0.1	109
		2027	38	0.2	114
		2028	70	0.1	164
		2029	37	0.1	131
		2030	38	0.1	126
		2031	60	0.1	131
		2032	74	0.2	156

Appendix 3a (cont.) Biochemical Parameters Measured in Bronchoalveolar Lavage (BAL)

Group	Sacrifice (weeks)	Animal No.	LDH [U/l]	β-Glucoronidase [U/l]	Total Protein [mg/l]
Carbon fiber med	1	3016	85	0.4	199
		3017	64	0.3	150
		3018	64	0.3	144
		3019	78	0.4	199
		3020	158	0.5	220
	8	3021	51	0.3	169
		3022	67	0.3	152
		3023	80	0.4	203
		3024	72	0.2	162
		3025	61	0.3	173
	15	3026	54	0.2	154
		3027	47	0.3	184
		3028	54	0.2	171
		3029	57	0.3	218
		3030	44	0.1	149
		3031	78	0.3	182
		3032	197	0.3	198
Carbon fiber high	1	4016	100	0.7	184
		4017	101	0.8	191
		4018	116	0.6	208
		4019	183	0.9	224
		4020	214	0.7	212
	8	4021	64	0.4	180
		4022	107	0.5	203
		4023	196	0.2	227
		4024	103	0.5	229
		4025	141	0.0	243
	15	4026	78	0.2	202
		4027	69	0.4	237
		4028	74	0.2	204
		4029	52	0.3	154
		4030	59	0.2	185
		4031	142	0.5	240
		4032	289	0.5	220

Appendix 3b Results of Differential Cell Count in BAL

Group	Sacrifice (weeks)	Animal No.	Cell concentration [cells/ml]	Macropages [%]	Eosinophil PMNs [%]	Neutrophil PMNs [%]	Lymphocytes [%]	Cell Viability
Control	1	16	160000	99.3	0.0	0.8	0.0	99
		17	205000	99.8	0.0	0.0	0.3	99
		18	96250	99.0	0.0	0.5	0.5	100
		19	135000	96.3	0.0	2.3	1.5	99
		20	120000	99.5	0.0	0.3	0.3	99
	8	21	131250	99.5	0.0	0.5	0.0	98
		22	147500	99.0	0.0	0.3	0.8	96
		23	152500	99.5	0.0	0.3	0.3	98
		24	160000	99.5	0.0	0.3	0.3	99
		25	128750	99.8	0.0	0.3	0.0	98
	15	26	175000	98.5	0.0	0.5	1.0	98
		27	165000	99.0	0.0	0.3	0.8	100
		28	182500	99.3	0.0	0.3	0.5	99
		29	145000	98.8	0.0	0.0	1.3	99
		30	125000	97.8	0.0	1.3	1.0	99
		32	137500	99.3	0.0	0.5	0.3	100
Carbon fiber low	1	16	156250	94.8	0.0	4.5	0.8	100
		17	142500	96.5	0.0	3.0	0.5	100
		18	146250	92.5	0.0	3.8	3.8	99
		19	142500	96.0	0.3	2.3	1.5	100
		20	155000	96.0	0.0	1.5	2.5	99
	8	21	116250	98.3	0.0	0.5	1.3	99
		22	177500	97.8	0.0	1.3	1.0	97
		23	153750	95.8	0.3	2.8	1.3	100
		24	160000	97.3	0.0	1.5	1.3	99
		25	117500	97.5	0.3	1.3	1.0	99
	15	26	143750	96.0	0.0	2.5	1.5	98
		27	148750	96.5	0.3	1.5	1.8	100
		28	230000	97.0	0.0	1.8	1.3	99
		29	122500	97.8	0.0	1.5	0.8	100
		30	106250	96.5	0.0	2.0	1.5	99
		31	166250	96.8	0.0	3.3	0.0	98
		32	167500	97.5	0.0	2.0	0.5	99

Appendix 3b (cont.) Results of Differential Cell Counts in BAL

Group	Sacrifice (weeks)	Animal No.	Cell concentration [cells/ml]	Macropages [%]	Eosinophil PMNs [%]	Neutrophil PMNs [%]	Lymphocytes [%]	Cell Viability
Carbon fiber med	1	16	215000	84.8	0.0	9.0	6.3	99
		17	172500	86.0	0.0	8.5	5.5	97
		18	201250	87.8	0.0	8.3	4.0	99
		19	161250	81.5	0.0	8.3	10.3	100
		20	93750	82.5	0.0	9.3	8.3	99
	8	21	196250	92.8	0.0	4.0	3.3	95
		22	106250	86.3	0.0	12.3	1.5	99
		23	187500	85.5	0.0	5.3	9.3	100
		24	163750	94.5	0.0	3.0	2.5	99
		25	227500	95.5	0.0	2.3	2.3	100
	15	26	191250	93.8	0.0	1.5	4.8	99
		27	200000	91.0	0.0	5.5	3.5	99
		28	260000	92.5	0.0	5.3	2.3	100
		29	140000	92.3	0.0	4.0	3.8	100
		30	138750	96.3	0.0	3.0	0.8	100
		31	166250	95.3	0.0	2.5	2.3	99
		32	167500	94.0	0.0	3.8	2.3	98
Carbon fiber high	1	16	132500	90.0	0.0	7.5	2.5	99
		17	145000	74.8	0.0	17.0	8.3	95
		18	216250	71.8	0.0	11.8	16.5	100
		19	171250	84.8	0.0	7.0	8.3	100
		20	148750	80.8	0.0	12.0	7.3	100
	8	21	191250	91.5	0.3	4.8	3.5	98
		22	176250	93.0	0.0	4.3	2.8	97
		23	262500	96.0	0.0	2.5	1.5	100
		24	187500	86.3	0.0	8.5	5.3	99
		25	195000	87.3	0.0	6.5	6.3	99
	15	26	201250	93.5	0.0	2.5	4.0	98
		27	267500	90.3	0.0	5.5	4.3	98
		28	197500	94.3	0.0	3.3	2.5	100
		29	111250	90.0	0.0	6.0	4.0	100
		30	212500	93.8	0.0	4.8	1.5	99
		31	212500	91.8	0.0	4.5	3.8	99
		32	160000	82.0	0.0	13.5	4.5	99

Appendix 4 Fiber and Particle Concentration in Aerosol Samples (SD: standard deviation)

Group	Date	Fibers	WHO fibers	Fibers L<5µm	Fibers L=5-20µm	Fibers L>20µm	Particles	Estimated mass conc. Fibers	Estimated mass conc. Particles	Grav. Conc.
Carbon	15/08/02					15.6				2.6
Fiber	16/08/02	105.6	62.6	43.0	41.7	20.9	21.5	2.7	0.4	3.2
low	19/08/02					12.9				2.7
	20/08/02					19.5				2.1
	21/08/02					19.2				3.4
	22/08/02					16.4				2.9
	23/08/02									3.1
	26/08/02									2.8
	27/08/02	71.3	42.3	29.0	25.5	16.8	19.1	2.3	0.2	2.9
	28/08/02									2.9
	29/08/02	66.0	54.2	11.9	35.5	18.6	1.6	2.0	0.1	2.5
	30/08/02									2.5
	02/09/02									3.0
	03/09/02					24.6				3.3
	04/09/02									2.5
	05/09/02	48.9	41.8	7.1	28.7	13.1	0.8	1.6	0.1	2.0
	06/09/02									2.1
	09/09/02									1.8
	10/09/02					10.9				2.4
	11/09/02									3.0
	12/09/02					14.8				2.0
	13/09/02									2.3
	16/09/02									2.0
	17/09/02	52.4	42.9	9.5	29.8	13.1	1.9	1.5	0.1	2.5
	18/09/02									1.5
	19/09/02					16.8				2.5
	20/09/02									2.0
	23/09/02									1.6
	24/09/02	69.2	56.8	12.4	41.5	15.3	3.3	1.8	0.1	1.5
	25/09/02									2.5
	26/09/02					12.9				2.2
	27/09/02									1.7
	30/09/02									2.5
	01/10/02	63.7	52.2	11.5	36.3	15.9	1.9	2.0	0.0	2.7
	02/10/02									2.0
	04/10/02					11.5				1.1
	07/10/02									1.9
	08/10/02	61.6	50.7	11.0	36.2	14.5	1.7	1.6	0.1	1.3
	09/10/02									1.2
	10/10/02					11.4				2.2
	11/10/02									1.3
	14/10/02									1.3
	15/10/02	60.8	50.2	10.6	35.7	14.5	3.4	1.6	0.1	2.3
	16/10/02									2.0
	17/10/02									1.8
	18/10/02									0.6
	21/10/02					14.1				2.4
	22/10/02									3.0
	23/10/02	77.9	62.6	15.3	44.2	18.4	5.2	1.8	0.1	2.3
	24/10/02									2.1
	25/10/02					13.1				2.6
	28/10/02									3.8
	29/10/02									1.5
	30/10/02	59.1	47.2	11.9	34.1	13.1	3.6	2.5	0.0	1.8
	31/10/02									1.3
	01/11/02	29.9	24.1	5.7	17.0	7.2	1.8	0.7	0.0	1.0
	04/11/02									0.8
	05/11/02									0.8
	06/11/02									0.9
	07/11/02					12.8				1.9
	08/11/02									2.0
	11/11/02	68.1	43.6	24.5	30.4	13.2	15.1	1.5	0.2	1.8
	12/11/02									1.8
	13/11/02					13.4				2.2
	14/11/02									1.8
	Mean	64.2	48.6	15.6	33.6	15.0	6.2	1.8	0.1	2.1

Appendix 4 (cont.) Fiber and Particle Concentration in Aerosol Samples (SD: standard deviation)

Group	Date	Fibers	WHO fibers	Fibers L<5µm	Fibers L=5-20µm	Fibers L>20µm	Particles	Estimated mass conc. Fibers	Estimated mass conc. Particles	Grav. Conc.
Carbon	15/08/02					63.0				7.4
Fiber	16/08/02	311.5	198.8	112.7	138.2	60.6	39.8	8.8	0.6	7.3
Med	19/08/02					50.1				7.4
	20/08/02					59.4				7.8
	21/08/02					36.7				7.0
	22/08/02					39.7				6.9
	23/08/02									6.2
	26/08/02									6.3
	27/08/02	226.0	145.2	80.8	84.2	61.0	74.4	7.2	0.4	7.5
	28/08/02									6.5
	29/08/02	172.5	143.1	29.4	97.8	45.3	5.1	4.9	0.3	6.4
	30/08/02									7.5
	02/09/02									6.6
	03/09/02					57.5				6.6
	04/09/02									5.4
	05/09/02	252.3	213.2	39.1	137.0	76.2	8.3	9.2	0.2	9.6
	06/09/02									6.7
	09/09/02									7.0
	10/09/02					49.2				6.6
	11/09/02									6.4
	12/09/02					45.7				6.8
	13/09/02									6.5
	16/09/02									6.2
	17/09/02	158.7	123.7	34.9	90.0	33.7	9.6	5.0	0.2	5.3
	18/09/02									7.0
	19/09/02					43.9				6.8
	20/09/02									6.2
	23/09/02									6.6
	24/09/02	207.0	179.3	27.8	123.9	55.4	2.4	6.4	0.3	6.2
	25/09/02									7.3
	26/09/02					53.1				6.4
	27/09/02									6.2
	30/09/02									6.3
	01/10/02	177.8	150.9	27.0	100.7	50.1	2.6	6.7	0.2	5.6
	02/10/02									6.1
	04/10/02					40.3				6.2
	07/10/02									6.1
	08/10/02	220.1	179.0	41.1	123.7	55.3	12.5	6.6	0.2	6.7
	09/10/02									6.7
	10/10/02					35.3				6.3
	11/10/02									6.2
	14/10/02									6.2
	15/10/02	184.3	148.7	35.6	104.5	44.2	9.1	5.4	0.2	5.6
	16/10/02									6.5
	17/10/02									6.3
	18/10/02									7.7
	21/10/02					75.4				10.6
	22/10/02									7.9
	23/10/02	263.8	210.9	52.9	155.6	55.3	13.6	6.4	0.3	6.8
	24/10/02									6.1
	25/10/02					41.6				6.9
	28/10/02									7.1
	29/10/02									6.3
	30/10/02	239.5	180.0	59.5	130.3	49.7	17.8	6.8	0.2	6.6
	31/10/02									6.9
	01/11/02	194.0	149.8	44.2	108.1	41.7	17.4	4.4	0.2	6.2
	04/11/02									6.7
	05/11/02									4.9
	06/11/02									8.0
	07/11/02					44.8				6.8
	08/11/02									6.4
	11/11/02	317.4	180.5	136.9	121.4	59.1	74.3	9.1	0.3	6.8
	12/11/02									7.6
	13/11/02					48.2				7.2
	14/11/02									7.1
	Mean	225.0	169.5	55.5	116.6	50.7	22.1	6.7	0.3	6.7

Appendix 4 (cont.) Fiber and Particle Concentration in Aerosol Samples (SD: standard deviation)

Group	Date	Fibers	WHO fibers	Fibers L<5µm	Fibers L=5-20µm	Fibers L>20µm	Particles	Estimated mass conc. Fibers	Estimated mass conc. Particles	Grav. Conc.
Carbon	15/08/02					163.2				23.7
Fiber	16/08/02	842.4	537.1	305.4	362.0	175.1	158.3	24.2	0.8	22.1
High	19/08/02					168.6				24.1
	20/08/02					205.1				24.0
	21/08/02					118.8				21.7
	22/08/02					155.5				21.4
	23/08/02									14.5
	26/08/02									22.8
	27/08/02	601.6	400.7	200.9	274.9	125.9	151.3	21.8	0.9	20.0
	28/08/02									23.3
	29/08/02	683.7	576.1	107.7	397.4	178.7	20.5	27.5	0.8	22.5
	30/08/02									23.3
	02/09/02									25.0
	03/09/02					181.5				23.5
	04/09/02									20.1
	05/09/02	634.7	572.9	61.8	373.3	199.6	18.0	28.3	0.5	21.4
	06/09/02									20.0
	09/09/02									18.7
	10/09/02					149.4				22.4
	11/09/02									22.2
	12/09/02					110.4				20.6
	13/09/02									17.2
	16/09/02									18.8
	17/09/02	514.3	438.6	75.7	291.5	147.1	9.3	15.7	0.4	16.8
	18/09/02									19.5
	19/09/02					129.3				19.1
	20/09/02									19.3
	23/09/02									21.3
	24/09/02	665.4	548.6	116.8	383.8	164.8	41.1	18.6	0.7	22.2
	25/09/02									22.2
	26/09/02					161.4				18.2
	27/09/02									23.7
	30/09/02									21.0
	01/10/02	760.3	461.0	299.3	312.1	148.9	206.1	19.6	1.3	21.8
	02/10/02									19.1
	04/10/02					147.2				19.8
	07/10/02									25.3
	08/10/02	541.5	445.7	95.8	300.4	145.3	13.8	20.0	1.0	20.1
	09/10/02									18.9
	10/10/02					141.1				22.0
	11/10/02									22.8
	14/10/02									22.8
	15/10/02	598.7	464.6	134.1	335.8	128.8	34.9	16.7	0.6	13.7
	16/10/02									15.3
	17/10/02									24.7
	18/10/02									24.1
	21/10/02					143.3				19.7
	22/10/02									19.2
	23/10/02	869.4	687.3	182.1	485.1	202.2	69.1	22.9	0.5	21.0
	24/10/02									18.8
	25/10/02					138.5				19.2
	28/10/02									19.1
	29/10/02									19.7
	30/10/02	835.5	654.6	180.8	472.7	181.9	50.8	24.1	1.2	19.4
	31/10/02									16.8
	01/11/02	711.4	563.9	147.5	393.4	170.5	46.6	17.6	1.4	20.8
	04/11/02									21.2
	05/11/02									19.7
	06/11/02									16.7
	07/11/02					120.0				19.3
	08/11/02									21.6
	11/11/02	758.0	553.0	205.0	340.7	212.3	138.4	22.6	2.1	20.7
	12/11/02									21.1
	13/11/02					176.5				19.7
	14/11/02									21.1
	Mean	693.6	531.1	162.5	363.3	158.3	73.7	21.5	0.9	20.6

Appendix 5a Fiber Size Distribution of Aerosol Samples

Group	Date	Fiber length [µm]			Fiber diameter [µm]			Estimated aerod. diameter [µm]		
		10%<	50%<	90%<	10%<	50%<	90%<	10%<	50%<	90%<
All fibers (L/D>3)										
Carbon Fiber Low	16/08/02	2.1	6.5	33.4	0.35	0.81	1.76	3.0	6.1	13.1
	27/08/02	2.1	7.6	34.7	0.40	0.82	1.89	3.4	6.7	18.1
	29/08/02	3.6	11.1	40.2	0.42	0.98	2.01	3.0	5.3	7.7
	05/09/02	4.2	11.9	36.2	0.45	0.99	2.14	3.0	5.5	10.1
	17/09/02	4.0	10.5	30.7	0.45	0.91	1.79	2.9	5.4	11.5
	24/09/02	3.6	10.1	34.3	0.50	0.96	1.85	2.9	5.2	9.3
	01/10/02	3.9	10.5	33.3	0.46	0.96	1.99	3.2	5.8	8.3
	08/10/02	3.7	10.5	32.5	0.47	0.93	1.88	2.8	5.1	9.2
	15/10/02	3.7	10.8	31.6	0.49	0.94	1.92	2.9	5.5	9.5
	23/10/02	3.6	10.4	31.3	0.45	0.86	1.84	2.8	5.4	8.3
	30/10/02	3.6	11.1	34.6	0.46	0.85	1.77	3.4	11.1	14.9
	01/11/02	3.1	9.8	30.7	0.40	0.91	1.84	2.9	5.6	10.3
	11/11/02	1.9	7.7	32.2	0.36	0.80	1.77	2.9	5.4	8.7
	Mean	3.3	9.9	33.5	0.43	0.90	1.88	3.0	6.0	10.7
	SD	0.8	1.6	2.6	0.05	0.07	0.11	0.2	1.6	3.0
Carbon Fiber Med	16/08/02	2.2	7.1	32.8	0.39	0.84	1.90	3.1	6.1	10.7
	27/08/02	2.4	8.0	42.8	0.36	0.77	1.89	3.2	6.3	12.4
	29/08/02	3.6	11.6	31.9	0.49	0.94	2.00	2.9	5.8	8.8
	05/09/02	3.9	14.0	41.1	0.49	0.95	2.08	3.0	5.6	9.0
	17/09/02	3.6	9.4	28.4	0.49	0.99	1.94	3.3	5.6	14.2
	24/09/02	4.2	11.0	37.7	0.50	1.04	1.89	3.1	5.1	13.6
	01/10/02	4.2	11.0	41.4	0.47	0.99	2.18	3.2	6.1	9.5
	08/10/02	3.7	10.7	38.3	0.45	1.03	2.19	3.0	6.2	9.7
	15/10/02	3.4	11.1	32.7	0.49	0.95	1.96	3.0	5.6	9.6
	23/10/02	3.4	9.8	28.6	0.45	0.90	1.92	2.8	5.3	10.2
	30/10/02	3.3	8.5	29.8	0.39	0.94	1.83	3.3	6.7	12.8
	01/11/02	3.6	9.2	32.2	0.43	0.88	1.78	2.7	5.6	12.1
	11/11/02	1.8	6.5	30.4	0.33	0.81	1.83	3.3	7.3	20.2
	Mean	3.3	9.8	34.5	0.44	0.92	1.95	3.1	5.9	11.8
	SD	0.7	2.0	5.1	0.06	0.08	0.13	0.2	0.6	3.1
Carbon Fiber High	16/08/02	2.2	7.6	35.0	0.40	0.83	1.77	3.0	6.9	13.7
	27/08/02	2.2	7.6	31.3	0.40	0.92	1.88	3.4	8.1	14.5
	29/08/02	3.9	10.5	37.4	0.47	0.94	1.93	3.3	6.2	11.8
	05/09/02	5.0	13.5	46.3	0.49	1.04	2.15	3.3	6.6	10.0
	17/09/02	4.2	11.7	42.6	0.46	0.95	2.08	2.9	5.3	8.5
	24/09/02	3.9	10.7	36.1	0.47	0.97	1.90	3.0	5.5	8.9
	01/10/02	2.2	7.0	36.5	0.36	0.76	1.83	3.2	5.8	10.1
	08/10/02	3.9	10.7	38.4	0.49	0.94	2.00	3.3	6.4	9.8
	15/10/02	3.4	9.5	33.4	0.49	0.98	1.88	2.9	5.7	10.7
	23/10/02	3.4	10.2	34.9	0.44	0.94	1.88	2.8	5.3	8.9
	30/10/02	3.3	10.1	32.4	0.42	0.93	1.93	3.0	5.0	8.8
	01/11/02	3.4	10.7	31.5	0.45	0.94	1.87	2.8	5.0	10.4
	11/11/02	2.8	10.2	36.7	0.39	0.82	1.93	3.1	6.0	11.3
	Mean	3.4	10.0	36.3	0.44	0.92	1.93	3.1	6.0	10.6
	SD	0.8	1.8	4.3	0.04	0.08	0.10	0.2	0.9	1.9

SD: Standard deviation

Appendix 5a (cont.) Fiber Size Distribution of Aerosol Samples

Group	Date	Fiber length [µm]			Fiber diameter [µm]			Estimated aerod. diameter [µm]		
		10%<	50%<	90%<	10%<	50%<	90%<	10%<	50%<	90%<
Fibers (L>20µm)										
Carbon Fiber Low	16/08/02	20.8	33.8	70.2	0.60	1.15	2.33	3.5	7.1	14.4
	27/08/02	21.1	32.8	78.7	0.50	1.22	2.61	3.8	6.8	18.1
	29/08/02	22.7	33.5	79.3	0.63	1.19	2.31	3.3	5.5	7.7
	05/09/02	22.1	31.6	96.8	0.49	1.06	2.26	3.2	5.9	10.2
	17/09/02	21.7	27.6	67.7	0.49	1.06	1.79	3.2	6.8	11.5
	24/09/02	20.5	33.4	71.5	0.56	1.10	2.47	3.1	5.8	9.4
	01/10/02	20.9	31.3	77.8	0.62	1.20	2.48	3.4	6.3	8.3
	08/10/02	21.4	28.6	61.3	0.50	1.09	2.38	3.3	5.5	9.5
	15/10/02	20.9	28.9	64.3	0.55	1.25	2.52	3.4	5.8	9.7
	23/10/02	20.6	28.1	65.9	0.59	1.12	2.38	3.1	5.6	8.3
	30/10/02	21.5	32.8	70.4	0.61	1.16	2.24	4.1	11.1	14.9
	01/11/02	20.9	29.1	67.7	0.53	1.02	2.39	3.0	6.4	10.6
	11/11/02	23.3	32.7	71.7	0.58	1.13	2.29	3.3	5.7	8.1
	Mean	21.4	31.1	72.6	0.56	1.13	2.34	3.4	6.5	10.8
	SD	0.8	2.3	9.1	0.05	0.07	0.20	0.3	1.5	3.1
Carbon Fiber Med	16/08/02	21.5	33.5	80.8	0.63	1.32	2.84	3.7	7.3	11.1
	27/08/02	21.8	34.6	86.1	0.53	1.12	2.39	3.4	7.0	12.4
	29/08/02	21.2	28.3	64.6	0.50	1.07	2.25	3.5	5.9	9.3
	05/09/02	21.8	31.5	109.3	0.63	1.10	2.29	3.2	5.7	9.0
	17/09/02	20.8	27.8	89.1	0.60	1.28	2.57	3.7	7.1	14.2
	24/09/02	22.3	31.8	72.4	0.66	1.20	2.77	3.2	6.7	13.6
	01/10/02	23.2	32.7	91.4	0.60	1.12	2.74	3.5	7.3	10.5
	08/10/02	21.2	34.0	59.8	0.61	1.04	2.38	3.1	6.8	12.7
	15/10/02	21.7	29.1	67.1	0.69	1.20	2.39	3.3	6.8	9.6
	23/10/02	22.0	28.3	65.0	0.60	1.12	2.57	3.3	6.3	10.2
	30/10/02	20.5	29.2	54.9	0.60	1.35	2.66	3.6	7.0	12.8
	01/11/02	21.4	32.1	53.1	0.60	0.99	2.27	3.0	6.4	12.1
	11/11/02	22.3	32.1	73.5	0.66	1.28	2.46	3.8	8.3	20.2
	Mean	21.7	31.1	74.4	0.61	1.17	2.51	3.4	6.8	12.1
	SD	0.7	2.3	16.3	0.05	0.11	0.20	0.2	0.7	3.0
Carbon Fiber high	16/08/02	23.5	34.7	76.9	0.54	1.03	2.03	3.4	9.7	13.7
	27/08/02	20.8	30.3	85.5	0.71	1.24	2.77	4.1	8.5	14.5
	29/08/02	21.8	30.7	100.9	0.60	1.28	2.27	3.8	7.4	11.8
	05/09/02	21.1	33.8	95.0	0.50	1.16	2.60	3.9	7.0	10.0
	17/09/02	21.2	33.3	72.4	0.65	1.13	2.66	3.3	5.7	8.5
	24/09/02	21.1	29.5	72.1	0.53	1.11	2.19	3.4	6.0	9.2
	01/10/02	23.0	36.5	86.1	0.55	1.19	2.32	3.4	6.0	10.1
	08/10/02	21.8	31.8	76.9	0.56	1.20	2.57	3.7	6.5	11.0
	15/10/02	21.7	31.3	73.2	0.74	1.13	2.47	3.1	6.0	12.0
	23/10/02	21.7	32.2	75.1	0.58	1.09	2.18	3.2	6.0	9.0
	30/10/02	21.8	31.2	76.6	0.71	1.36	2.78	3.4	5.5	11.2
	01/11/02	21.4	28.9	59.4	0.55	1.28	2.37	3.5	6.0	12.7
	11/11/02	21.2	31.1	68.5	0.58	1.14	2.45	3.3	6.6	11.3
	Mean	21.7	32.0	78.4	0.60	1.18	2.44	3.5	6.7	11.2
	SD	0.8	2.1	11.1	0.08	0.09	0.23	0.3	1.2	1.8

SD: Standard deviation

Appendix 5a (cont.) Fiber Size Distribution of Aerosol Samples

Group	Date	Fiber length [µm]			Fiber diameter [µm]			Estimated aerod. diameter [µm]		
		10%<	50%<	90%<	10%<	50%<	90%<	10%<	50%<	90%<
WHO fibers										
Carbon Fiber Low	16/08/02	5.8	12.2	42.3	0.50	1.02	1.98	2.9	5.5	8.4
	27/08/02	6.5	15.4	43.3	0.50	1.14	2.03	3.0	5.4	7.1
	29/08/02	5.9	14.1	44.7	0.49	1.07	2.00	2.8	5.2	7.0
	05/09/02	6.5	13.4	38.0	0.49	1.09	2.06	2.8	4.7	7.1
	17/09/02	6.2	13.2	33.0	0.49	1.00	1.84	2.6	4.3	6.0
	24/09/02	6.5	12.0	37.0	0.53	1.04	1.85	2.7	4.5	6.5
	01/10/02	6.1	12.5	35.8	0.51	1.02	2.00	3.2	5.7	7.6
	08/10/02	6.2	12.9	35.3	0.52	0.99	1.88	2.6	4.4	6.3
	15/10/02	5.9	12.9	36.5	0.50	1.00	1.88	2.8	4.7	7.4
	23/10/02	5.9	12.5	35.0	0.49	0.98	1.86	2.7	4.7	7.0
	30/10/02	5.9	13.5	38.0	0.49	0.93	1.78	2.6	4.4	6.1
	01/11/02	6.2	11.9	35.0	0.43	1.00	1.86	2.7	4.5	7.6
	11/11/02	6.5	12.6	36.4	0.51	1.09	1.95	2.9	4.9	7.2
	Mean	6.2	13.0	37.7	0.50	1.03	1.92	2.8	4.8	7.0
	SD	0.3	1.0	3.6	0.02	0.06	0.09	0.2	0.5	0.7
Carbon Fiber Med	16/08/02	5.8	12.9	42.3	0.53	1.04	1.99	2.9	4.9	7.3
	27/08/02	6.2	16.2	53.4	0.53	1.04	2.03	3.0	5.6	7.7
	29/08/02	6.2	13.7	33.5	0.50	0.98	2.00	2.8	5.1	6.6
	05/09/02	6.8	15.6	49.1	0.55	1.06	2.00	3.0	5.2	6.7
	17/09/02	5.9	12.0	32.8	0.53	1.10	1.99	3.1	4.9	8.6
	24/09/02	5.9	13.2	39.8	0.55	1.11	1.88	2.8	4.5	7.2
	01/10/02	6.4	12.6	43.8	0.53	1.06	2.17	2.9	5.4	8.4
	08/10/02	6.4	13.5	39.5	0.50	1.13	2.20	2.9	5.3	7.5
	15/10/02	6.4	13.1	39.2	0.55	1.06	1.97	2.9	5.1	9.6
	23/10/02	6.1	11.9	32.5	0.54	1.00	1.92	2.7	4.6	7.1
	30/10/02	6.1	12.3	34.9	0.50	1.11	1.89	2.9	4.9	7.0
	01/11/02	5.8	11.9	33.8	0.49	0.98	1.87	2.5	4.8	8.4
	11/11/02	6.4	12.5	41.6	0.53	1.13	2.02	2.9	5.3	8.3
	Mean	6.2	13.2	39.7	0.53	1.06	2.00	2.9	5.0	7.7
	SD	0.3	1.3	6.5	0.02	0.05	0.10	0.2	0.3	0.9
Carbon Fiber High	16/08/02	6.1	12.3	43.0	0.55	1.04	1.88	2.6	4.2	6.3
	27/08/02	6.1	12.5	39.3	0.54	1.09	1.91	3.0	5.4	9.0
	29/08/02	6.1	12.9	40.1	0.50	1.05	1.97	3.0	4.9	6.8
	05/09/02	6.4	14.8	50.3	0.50	1.09	2.04	3.0	5.8	10.0
	17/09/02	6.4	13.7	44.5	0.53	1.04	2.02	2.8	4.8	7.1
	24/09/02	6.5	13.2	38.4	0.49	1.02	1.93	2.9	4.9	7.5
	01/10/02	6.2	12.8	46.9	0.49	1.06	2.00	3.0	5.3	7.4
	08/10/02	6.4	13.1	47.5	0.53	1.02	2.03	3.0	5.9	8.3
	15/10/02	6.4	12.9	35.8	0.60	1.06	1.96	2.8	5.4	8.2
	23/10/02	6.1	13.1	39.9	0.54	1.02	1.92	2.8	5.2	6.9
	30/10/02	6.1	12.3	38.6	0.50	1.09	1.98	2.9	4.7	7.3
	01/11/02	6.5	13.4	34.3	0.50	1.07	1.87	2.6	4.3	6.2
	11/11/02	6.2	14.7	42.9	0.49	0.97	1.98	2.8	4.9	7.1
	Mean	6.3	13.2	41.7	0.52	1.05	1.96	2.9	5.0	7.5
	SD	0.2	0.8	4.7	0.03	0.03	0.06	0.2	0.5	1.1

SD: Standard deviation

Appendix 5a (cont.) Fiber Size Distribution of Aerosol Samples

Group	Date	Fiber length [µm]				Fiber diameter [µm]			
		Arithmetic		Geometric		Arithmetic		Geometric	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
All Fibers (L/D > 3)									
Carbon Fiber Low	16/08/02	13.39	19.58	7.42	2.81	0.96	0.65	0.80	1.82
	27/08/02	15.58	25.47	8.10	3.00	1.03	0.76	0.83	1.88
	29/08/02	18.41	21.34	11.64	2.55	1.11	0.64	0.94	1.77
	05/09/02	18.48	21.88	12.32	2.36	1.17	0.69	0.99	1.79
	17/09/02	16.88	23.80	11.11	2.35	1.05	0.58	0.91	1.70
	24/09/02	15.51	16.57	10.63	2.32	1.13	0.66	0.98	1.69
	01/10/02	17.57	24.32	11.31	2.39	1.13	0.66	0.97	1.75
	08/10/02	16.31	23.01	11.02	2.29	1.09	0.64	0.93	1.73
	15/10/02	15.93	16.10	11.25	2.26	1.12	0.67	0.96	1.73
	23/10/02	15.42	16.59	10.57	2.33	1.05	0.63	0.90	1.72
	30/10/02	17.21	28.60	10.85	2.45	1.02	0.64	0.88	1.72
	01/11/02	15.83	19.08	10.46	2.44	1.04	0.62	0.88	1.80
	11/11/02	13.58	17.32	7.86	2.82	0.97	0.66	0.79	1.87
	Mean	16.16	21.05	10.35	2.49	1.07	0.65	0.91	1.77
	SD	1.53	3.81	1.50	0.23	0.06	0.04	0.06	0.06
Carbon Fiber Med	16/08/02	13.78	17.85	7.99	2.74	1.03	0.70	0.85	1.84
	27/08/02	16.99	22.85	9.00	3.04	0.98	0.69	0.80	1.88
	29/08/02	17.13	23.65	11.42	2.35	1.08	0.64	0.93	1.73
	05/09/02	22.11	30.72	13.57	2.54	1.15	0.69	0.98	1.75
	17/09/02	15.75	24.52	9.92	2.38	1.12	0.66	0.97	1.70
	24/09/02	17.65	19.29	11.93	2.35	1.18	0.68	1.02	1.70
	01/10/02	19.22	22.57	12.43	2.43	1.17	0.69	1.00	1.75
	08/10/02	16.36	16.07	11.28	2.36	1.18	0.70	1.00	1.80
	15/10/02	16.24	18.02	11.03	2.37	1.10	0.63	0.95	1.71
	23/10/02	14.45	15.18	9.97	2.33	1.09	0.67	0.92	1.78
	30/10/02	14.15	16.22	9.37	2.43	1.07	0.65	0.90	1.80
	01/11/02	14.85	17.62	9.97	2.36	1.04	0.62	0.89	1.72
	11/11/02	12.97	19.54	6.90	2.94	0.97	0.73	0.78	1.95
	Mean	16.28	20.32	10.37	2.51	1.09	0.67	0.92	1.78
	SD	2.40	4.26	1.79	0.23	0.07	0.03	0.08	0.07
Carbon Fiber High	16/08/02	14.98	21.81	8.26	2.85	1.00	0.66	0.84	1.81
	27/08/02	14.71	21.23	8.39	2.76	1.07	0.72	0.89	1.86
	29/08/02	20.08	37.27	11.87	2.47	1.11	0.64	0.96	1.71
	05/09/02	21.70	27.30	14.13	2.39	1.22	0.74	1.03	1.77
	17/09/02	18.60	20.27	12.39	2.42	1.12	0.68	0.96	1.76
	24/09/02	17.60	25.08	11.47	2.35	1.10	0.64	0.95	1.71
	01/10/02	14.77	22.53	7.86	2.88	0.96	0.64	0.79	1.87
	08/10/02	18.92	29.03	11.75	2.48	1.11	0.68	0.95	1.76
	15/10/02	15.86	21.11	10.31	2.39	1.11	0.61	0.98	1.67
	23/10/02	16.53	20.03	10.62	2.50	1.07	0.58	0.93	1.71
	30/10/02	16.46	23.43	10.34	2.47	1.12	0.72	0.94	1.82
	01/11/02	15.88	19.10	10.83	2.33	1.08	0.64	0.93	1.72
	11/11/02	16.66	18.91	10.17	2.72	1.03	0.70	0.85	1.85
	Mean	17.13	23.62	10.65	2.54	1.09	0.66	0.92	1.77
	SD	2.09	4.98	1.72	0.19	0.06	0.04	0.06	0.06

Appendix 5a (cont.) Fiber Size Distribution of Aerosol Samples

Group	Date	Fiber length [µm]				Fiber diameter [µm]			
		Arithmetic		Geometric		Arithmetic		Geometric	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Fibers (L > 20 µm)									
Carbon Fiber Low	16/08/02	41.67	29.24	36.24	1.62	1.38	0.88	1.18	1.71
	27/08/02	44.10	39.99	36.27	1.71	1.47	1.01	1.23	1.81
	29/08/02	43.05	26.55	37.59	1.63	1.34	0.70	1.17	1.71
	05/09/02	42.82	30.27	36.25	1.70	1.27	0.78	1.06	1.85
	17/09/02	40.55	38.09	33.41	1.69	1.18	0.62	1.04	1.65
	24/09/02	39.24	21.29	35.00	1.57	1.36	0.83	1.16	1.74
	01/10/02	43.66	37.48	36.09	1.72	1.38	0.70	1.21	1.68
	08/10/02	39.79	38.26	33.48	1.64	1.29	0.82	1.09	1.77
	15/10/02	37.07	20.64	33.20	1.55	1.39	0.83	1.19	1.75
	23/10/02	37.07	22.05	32.70	1.59	1.31	0.79	1.13	1.72
	30/10/02	45.58	50.68	36.98	1.70	1.34	0.85	1.15	1.70
	01/11/02	38.65	27.57	33.71	1.60	1.25	0.76	1.07	1.75
	11/11/02	40.54	23.45	36.24	1.55	1.32	0.70	1.16	1.66
	Mean	41.06	31.20	35.17	1.64	1.33	0.79	1.14	1.73
	SD	2.62	8.83	1.61	0.06	0.07	0.10	0.06	0.05
Carbon Fiber Med	16/08/02	41.60	24.11	36.56	1.62	1.54	0.91	1.32	1.74
	27/08/02	44.41	28.93	38.04	1.69	1.33	0.86	1.12	1.79
	29/08/02	39.29	37.32	32.93	1.65	1.24	0.72	1.05	1.80
	05/09/02	49.88	44.27	39.35	1.86	1.27	0.71	1.11	1.66
	17/09/02	43.00	42.55	33.66	1.80	1.47	0.90	1.27	1.70
	24/09/02	40.74	24.56	35.76	1.61	1.44	0.93	1.23	1.73
	01/10/02	45.21	28.59	39.05	1.66	1.42	0.87	1.19	1.79
	08/10/02	38.13	18.08	35.00	1.49	1.27	0.76	1.10	1.67
	15/10/02	38.82	24.68	34.37	1.57	1.42	0.82	1.25	1.64
	23/10/02	36.54	20.04	32.93	1.52	1.38	0.83	1.18	1.73
	30/10/02	36.86	22.91	32.79	1.55	1.44	0.74	1.26	1.70
	01/11/02	38.04	25.98	33.55	1.56	1.28	0.79	1.10	1.71
	11/11/02	42.29	29.99	36.31	1.65	1.48	0.99	1.28	1.66
	Mean	41.14	28.62	35.41	1.63	1.38	0.83	1.19	1.72
	SD	3.72	7.91	2.26	0.10	0.09	0.08	0.08	0.05
Carbon Fiber High	16/08/02	45.46	32.12	39.14	1.64	1.25	0.77	1.08	1.69
	27/08/02	42.99	32.47	36.15	1.70	1.57	0.96	1.34	1.75
	29/08/02	51.09	62.86	37.98	1.89	1.36	0.75	1.19	1.66
	05/09/02	47.11	37.16	38.97	1.75	1.37	0.83	1.15	1.83
	17/09/02	41.81	25.01	36.91	1.60	1.38	0.75	1.20	1.68
	24/09/02	43.54	39.79	35.96	1.71	1.29	0.81	1.10	1.75
	01/10/02	47.66	33.94	40.41	1.70	1.34	0.74	1.17	1.68
	08/10/02	46.31	45.37	37.89	1.73	1.42	0.88	1.21	1.77
	15/10/02	42.07	33.36	35.66	1.66	1.44	0.83	1.26	1.64
	23/10/02	41.88	28.35	36.28	1.63	1.28	0.69	1.12	1.67
	30/10/02	44.55	37.77	37.16	1.69	1.55	0.94	1.35	1.66
	01/11/02	37.65	28.74	32.76	1.58	1.41	0.84	1.20	1.75
	11/11/02	39.22	22.45	34.99	1.56	1.39	0.86	1.19	1.73
	Mean	43.95	35.34	36.94	1.68	1.39	0.82	1.20	1.71
	SD	3.54	10.04	1.95	0.08	0.09	0.08	0.08	0.05

Appendix 5a (cont.) Fiber Size Distribution of Aerosol Samples

Group	Date	Fiber length [µm]				Fiber diameter [µm]			
		Arithmetic		Geometric		Arithmetic		Geometric	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
WHO Fibers									
Carbon Fiber Low	16/08/02	20.17	22.79	14.28	2.15	1.15	0.57	1.01	1.66
	27/08/02	23.96	30.62	16.65	2.16	1.22	0.60	1.07	1.69
	29/08/02	21.71	22.49	15.31	2.19	1.16	0.57	1.02	1.67
	05/09/02	20.54	22.19	15.03	2.05	1.19	0.61	1.03	1.73
	17/09/02	18.77	20.96	14.11	1.99	1.10	0.54	0.97	1.65
	24/09/02	17.98	17.29	13.59	1.98	1.15	0.53	1.04	1.60
	01/10/02	20.62	26.11	14.53	2.10	1.17	0.59	1.03	1.67
	08/10/02	18.83	24.69	13.93	1.99	1.12	0.56	0.99	1.66
	15/10/02	18.47	16.80	14.24	1.96	1.12	0.56	0.99	1.67
	23/10/02	18.28	17.44	13.89	1.98	1.10	0.57	0.96	1.66
	30/10/02	18.79	18.18	14.32	1.98	1.05	0.51	0.94	1.62
	01/11/02	18.51	19.92	13.88	2.00	1.10	0.55	0.96	1.72
	11/11/02	19.58	19.21	14.57	2.04	1.16	0.56	1.03	1.65
	Mean	19.71	21.44	14.49	2.05	1.14	0.56	1.00	1.66
	SD	1.65	3.90	0.78	0.08	0.04	0.03	0.04	0.03
Carbon Fiber Med	16/08/02	19.14	18.80	13.98	2.10	1.17	0.57	1.04	1.66
	27/08/02	24.57	25.32	17.09	2.26	1.16	0.61	1.02	1.69
	29/08/02	19.56	24.77	14.53	2.00	1.12	0.58	0.99	1.65
	05/09/02	25.57	32.69	17.43	2.18	1.16	0.56	1.04	1.64
	17/09/02	18.92	26.80	13.25	2.05	1.19	0.56	1.06	1.63
	24/09/02	19.71	19.94	14.36	2.10	1.18	0.54	1.06	1.62
	01/10/02	21.54	23.36	15.14	2.17	1.20	0.61	1.06	1.67
	08/10/02	19.22	16.53	14.78	1.99	1.25	0.63	1.09	1.70
	15/10/02	19.07	18.74	14.53	1.98	1.15	0.53	1.03	1.62
	23/10/02	17.02	15.73	13.15	1.95	1.14	0.56	1.01	1.64
	30/10/02	16.91	14.15	13.22	1.95	1.17	0.58	1.03	1.70
	01/11/02	17.95	18.70	13.52	2.00	1.09	0.56	0.96	1.65
	11/11/02	19.99	20.97	14.50	2.10	1.22	0.59	1.08	1.67
	Mean	19.94	21.27	14.57	2.06	1.17	0.58	1.04	1.66
	SD	2.52	5.02	1.32	0.09	0.04	0.03	0.04	0.03
Carbon Fiber High	16/08/02	20.65	21.26	14.81	2.14	1.15	0.53	1.04	1.58
	27/08/02	19.73	21.56	14.08	2.12	1.19	0.53	1.07	1.61
	29/08/02	21.67	30.91	14.67	2.15	1.15	0.56	1.02	1.66
	05/09/02	23.52	28.32	16.20	2.19	1.19	0.60	1.04	1.69
	17/09/02	21.05	21.00	15.37	2.10	1.14	0.58	1.01	1.68
	24/09/02	20.50	26.86	14.62	2.06	1.13	0.55	1.01	1.65
	01/10/02	22.21	26.34	15.08	2.23	1.17	0.57	1.04	1.67
	08/10/02	21.94	31.16	15.07	2.15	1.17	0.59	1.03	1.67
	15/10/02	19.25	22.78	14.07	2.03	1.18	0.54	1.06	1.59
	23/10/02	19.85	21.13	14.56	2.06	1.15	0.54	1.03	1.61
	30/10/02	20.02	25.54	14.16	2.09	1.20	0.59	1.06	1.68
	01/11/02	18.94	20.49	14.47	1.94	1.12	0.52	1.00	1.62
	11/11/02	21.32	19.75	15.83	2.09	1.12	0.59	0.98	1.70
	Mean	20.82	24.39	14.85	2.10	1.16	0.56	1.03	1.65
	SD	1.27	3.92	0.64	0.07	0.03	0.03	0.03	0.04

Appendix 5b Fiber Size Distribution of Lung Samples

Group	Sacrifice date [weeks]	Animal No.	Fiber length [µm]			Fiber diameter [µm]			Estimated aerodynamic diameter [µm]		
			10%<	50%<	90%<	10%<	50%<	90%<	10%<	50%<	90%<
All fibers (L/D>3)											
Carbon Fiber Low	1	1	2.7	7.3	25.8	0.38	0.85	1.69	0.8	1.7	3.5
		2	3.0	7.1	26.1	0.50	0.84	1.69	1.0	1.7	3.2
		3	3.6	9.5	28.5	0.58	0.97	1.74	1.2	2.0	3.6
		4	3.6	9.1	27.8	0.56	0.97	1.63	1.2	2.0	3.4
		5	3.6	10.0	31.9	0.53	0.91	1.68	1.1	2.0	3.5
	8	6	3.4	8.7	25.0	0.44	0.84	1.48	0.9	1.7	3.1
		7	4.4	12.4	34.3	0.53	0.93	1.65	1.2	2.0	3.5
		8	4.4	11.5	30.0	0.51	0.89	1.63	1.1	2.0	3.3
		9	4.9	10.5	30.5	0.49	0.89	1.55	1.1	1.9	3.2
		10	4.2	10.7	31.7	0.59	0.93	1.68	1.2	2.0	3.5
	15	11	4.6	13.5	38.0	0.54	0.92	1.57	1.2	2.0	3.3
		12	4.4	12.2	37.3	0.40	0.87	1.61	0.9	1.9	3.4
		13	4.4	11.4	35.3	0.49	0.93	1.53	1.1	2.0	3.3
		14	4.9	11.6	34.9	0.44	0.91	1.68	1.0	2.0	3.5
		15	4.9	13.9	37.2	0.51	0.92	1.65	1.1	2.1	3.5
Carbon Fiber Med	1	1	3.0	6.8	19.7	0.49	0.84	1.67	1.0	1.7	3.3
		2	3.6	8.2	23.6	0.52	0.89	1.66	1.1	1.9	3.4
		3	3.0	7.7	26.3	0.45	0.87	1.51	0.9	1.8	3.1
		4	3.3	9.5	26.1	0.50	0.95	1.71	1.1	2.0	3.4
		5	3.0	6.5	19.0	0.50	0.89	1.63	1.0	1.8	3.2
	8	6	3.6	10.7	27.5	0.42	0.80	1.54	0.9	1.8	3.3
		7	3.7	11.4	30.5	0.44	0.84	1.45	1.0	1.8	3.0
		8	4.6	11.2	26.7	0.45	0.91	1.72	1.1	2.0	3.6
		9	4.0	11.5	29.5	0.44	0.85	1.51	1.0	1.8	3.4
		10	3.6	10.3	28.8	0.45	0.87	1.58	1.0	1.9	3.2
	15	11	4.6	13.5	35.2	0.49	0.93	1.70	1.2	2.0	3.6
		12	4.0	10.8	31.7	0.48	0.87	1.60	1.0	1.9	3.4
		13	4.1	10.2	32.4	0.55	0.93	1.66	1.2	2.1	3.6
		14	5.0	12.3	34.3	0.49	0.88	1.60	1.1	1.9	3.3
		15	4.0	12.2	36.2	0.52	0.93	1.56	1.1	2.0	3.5
Carbon Fiber High	1	1	3.1	8.9	25.1	0.46	0.92	1.73	1.0	1.9	3.5
		2	2.8	8.2	26.6	0.45	0.88	1.61	0.9	1.8	3.3
		3	2.7	7.0	20.5	0.46	0.85	1.69	0.9	1.7	3.3
		4	2.4	6.4	22.1	0.40	0.87	1.58	0.8	1.7	3.1
		5	3.7	10.1	28.7	0.52	0.93	1.64	1.1	2.0	3.4
	8	6	3.3	8.6	27.9	0.44	0.82	1.48	0.9	1.8	3.0
		7	3.4	9.5	29.8	0.47	0.86	1.60	1.0	1.8	3.3
		8	3.4	9.5	29.9	0.41	0.87	1.60	0.9	1.9	3.2
		9	3.6	13.0	28.8	0.46	0.89	1.55	1.0	2.0	3.3
		10	3.3	8.7	29.8	0.45	0.87	1.50	0.9	1.9	3.2
	15	11	4.8	13.1	33.9	0.49	0.97	1.81	1.1	2.1	3.9
		12	5.2	13.0	33.7	0.46	0.97	1.74	1.1	2.1	3.6
		13	4.8	11.1	31.7	0.49	0.94	1.70	1.2	2.0	3.5
		14	3.8	10.6	31.9	0.45	0.91	1.81	1.0	2.0	3.7
		15	4.1	11.5	30.4	0.50	0.90	1.72	1.1	1.9	3.7

Appendix 5b (cont.) Fiber Size Distribution of Lung Samples

Group	Sacrifice date [weeks]	Animal No.	Fiber length [µm]			Fiber diameter [µm]			Estimated aerodynamic diameter [µm]		
			10%<	50%<	90%<	10%<	50%<	90%<	10%<	50%<	90%<
Fibers L > 20µm											
Carbon Fiber Low	1	1	21.8	27.9	49.3	0.58	1.05	1.75	1.5	2.6	4.1
		2	21.5	28.1	46.2	0.64	1.00	1.74	1.6	2.5	4.0
		3	21.5	29.0	49.9	0.69	1.20	1.95	1.7	2.9	4.2
		4	21.7	28.4	49.0	0.66	1.02	1.82	1.6	2.5	4.0
		5	22.4	30.0	51.1	0.59	1.09	1.64	1.6	2.7	3.9
	8	6	21.6	28.8	50.2	0.57	1.01	1.56	1.5	2.5	3.6
		7	21.7	30.3	52.4	0.54	1.01	1.66	1.5	2.5	3.9
		8	21.2	26.4	45.1	0.59	1.00	1.59	1.5	2.4	3.6
		9	20.6	29.0	51.6	0.57	0.93	1.59	1.5	2.4	3.7
		10	21.8	28.8	50.8	0.61	1.03	1.92	1.5	2.6	4.2
	15	11	21.3	29.9	48.5	0.61	0.97	1.71	1.6	2.4	3.9
		12	21.2	30.5	53.6	0.52	0.90	1.72	1.4	2.3	3.8
		13	22.5	31.0	50.9	0.54	1.07	1.55	1.5	2.6	3.6
		14	21.8	28.8	50.6	0.61	1.06	1.84	1.6	2.6	4.1
		15	20.8	29.6	49.6	0.62	1.03	1.98	1.6	2.5	4.4
Carbon Fiber Med	1	1	21.4	26.3	44.2	0.66	1.05	1.69	1.7	2.6	3.9
		2	22.1	29.2	46.0	0.60	0.99	1.77	1.6	2.4	4.2
		3	22.1	29.0	43.5	0.60	0.91	1.50	1.5	2.2	3.5
		4	22.0	30.0	48.8	0.60	0.95	2.07	1.5	2.4	4.6
		5	21.4	27.9	46.5	0.65	1.09	1.85	1.7	2.7	4.1
	8	6	21.0	26.8	44.8	0.53	0.89	1.66	1.4	2.2	3.9
		7	21.3	30.7	47.6	0.54	0.97	1.70	1.4	2.4	3.9
		8	21.7	25.9	39.5	0.45	1.01	2.01	1.2	2.4	4.3
		9	20.8	29.0	52.2	0.55	0.93	1.82	1.5	2.3	4.1
		10	21.0	27.9	53.4	0.48	0.89	1.56	1.3	2.2	3.5
	15	11	22.1	30.8	52.5	0.52	1.11	1.67	1.4	2.7	4.0
		12	23.0	29.6	46.5	0.63	1.03	1.76	1.6	2.5	4.0
		13	21.2	30.0	46.7	0.61	1.09	2.15	1.6	2.6	4.8
		14	21.0	30.7	50.8	0.54	0.89	1.51	1.4	2.2	3.5
		15	21.3	29.6	49.9	0.63	1.06	1.65	1.6	2.7	3.8
Carbon Fiber High	1	1	21.7	27.9	44.2	0.55	1.02	1.90	1.4	2.5	4.4
		2	20.8	28.7	45.9	0.54	0.99	1.72	1.4	2.4	4.1
		3	21.4	26.1	43.9	0.62	1.16	2.03	1.6	2.8	4.7
		4	22.1	27.3	46.5	0.54	0.95	1.81	1.4	2.4	4.2
		5	20.8	27.3	47.2	0.56	1.08	1.77	1.5	2.5	4.1
	8	6	21.3	29.4	47.2	0.54	0.93	1.66	1.4	2.3	3.7
		7	21.2	30.3	60.5	0.54	0.94	1.92	1.4	2.3	4.1
		8	21.6	29.4	54.3	0.47	0.87	1.72	1.3	2.2	4.0
		9	21.3	27.6	45.5	0.56	0.97	1.72	1.5	2.4	4.0
		10	21.6	29.6	52.9	0.49	0.93	1.64	1.3	2.3	3.8
	15	11	20.8	28.2	48.9	0.49	1.07	2.19	1.3	2.7	4.9
		12	22.4	31.2	51.7	0.56	1.04	1.77	1.5	2.5	4.2
		13	22.0	27.4	48.9	0.50	1.01	1.84	1.4	2.5	4.2
		14	21.7	29.2	48.7	0.59	0.96	1.87	1.5	2.4	4.2
		15	21.7	28.3	47.6	0.56	1.00	1.72	1.5	2.5	3.9

Appendix 5b (cont.) Fiber Size Distribution of Lung Samples

Group	Sacrifice date [weeks]	Animal No.	Fiber length [µm]			Fiber diameter [µm]			Estimated aerodynamic diameter [µm]		
			10%<	50%<	90%<	10%<	50%<	90%<	10%<	50%<	90%<
WHO Fibers											
Carbon Fiber Low	1	1	5.6	10.4	28.5	0.55	1.04	1.87	1.2	2.2	3.7
		2	5.6	10.5	29.4	0.53	1.00	1.79	1.2	2.2	3.3
		3	5.9	12.2	31.0	0.64	1.05	1.79	1.4	2.2	3.8
		4	5.8	11.3	30.4	0.65	1.04	1.77	1.5	2.2	3.6
		5	6.1	12.9	36.7	0.56	1.04	1.74	1.3	2.2	3.6
	8	6	6.0	10.3	27.1	0.49	0.93	1.55	1.1	2.0	3.3
		7	6.7	13.8	35.8	0.54	0.95	1.67	1.3	2.1	3.5
		8	6.6	13.9	31.7	0.54	0.97	1.65	1.3	2.2	3.4
		9	6.7	11.9	31.7	0.51	0.90	1.60	1.2	2.0	3.2
		10	6.1	11.9	33.3	0.61	0.97	1.71	1.4	2.1	3.6
	15	11	6.9	15.6	38.7	0.57	0.96	1.63	1.3	2.2	3.4
		12	6.7	14.2	40.1	0.49	0.94	1.68	1.2	2.1	3.6
		13	6.4	13.1	36.6	0.54	0.99	1.60	1.2	2.2	3.4
		14	6.7	13.6	37.3	0.50	0.97	1.70	1.2	2.2	3.5
		15	6.6	15.3	39.5	0.55	0.95	1.65	1.3	2.1	3.6
Carbon Fiber Med	1	1	5.8	9.2	23.0	0.56	1.02	1.80	1.3	2.2	3.5
		2	5.6	11.6	27.0	0.60	0.98	1.79	1.3	2.2	3.5
		3	5.8	11.0	29.1	0.55	0.95	1.62	1.3	2.1	3.3
		4	6.1	11.3	30.0	0.60	1.04	1.74	1.4	2.2	3.5
		5	5.8	10.4	25.2	0.61	1.06	1.80	1.4	2.2	3.6
	8	6	6.6	13.4	30.5	0.47	0.88	1.63	1.1	1.9	3.4
		7	6.2	13.4	32.5	0.48	0.93	1.52	1.1	2.0	3.2
		8	6.4	12.6	27.9	0.50	0.97	1.78	1.2	2.1	3.7
		9	6.0	13.5	32.7	0.47	0.90	1.63	1.1	2.0	3.5
		10	6.1	12.7	31.7	0.48	0.93	1.64	1.1	2.1	3.3
	15	11	7.0	14.8	37.3	0.55	1.00	1.73	1.3	2.2	3.6
		12	6.2	13.6	34.9	0.55	0.97	1.65	1.3	2.1	3.6
		13	6.2	12.8	33.2	0.57	0.97	1.70	1.3	2.1	3.6
		14	6.5	13.5	35.4	0.50	0.91	1.64	1.2	2.1	3.5
		15	6.6	14.4	39.5	0.61	1.01	1.62	1.4	2.2	3.5
Carbon Fiber High	1	1	5.9	11.0	27.6	0.56	1.04	1.85	1.3	2.2	3.7
		2	6.1	12.6	30.6	0.50	0.95	1.72	1.2	2.2	3.6
		3	5.6	9.7	24.1	0.54	1.02	1.90	1.3	2.2	3.7
		4	5.8	9.2	26.1	0.51	1.05	1.71	1.2	2.1	3.4
		5	6.4	12.6	31.2	0.58	1.02	1.77	1.3	2.2	3.5
	8	6	5.8	11.0	31.3	0.50	0.93	1.55	1.1	2.1	3.3
		7	5.8	11.4	33.3	0.51	0.97	1.66	1.2	2.1	3.5
		8	6.0	12.7	32.8	0.44	0.93	1.64	1.1	2.1	3.3
		9	6.7	15.3	31.6	0.52	0.97	1.59	1.2	2.2	3.4
		10	6.0	11.5	32.3	0.50	0.95	1.63	1.2	2.1	3.5
	15	11	6.7	14.2	34.5	0.49	1.02	1.83	1.2	2.3	3.9
		12	6.1	13.9	36.5	0.50	0.98	1.74	1.2	2.2	3.7
		13	6.5	12.6	33.2	0.52	0.99	1.73	1.3	2.2	3.6
		14	6.4	13.5	33.6	0.54	0.97	1.88	1.2	2.2	3.8
		15	6.2	13.0	32.7	0.54	0.97	1.86	1.3	2.2	3.7

Appendix 5b (cont.) Fiber Size Distribution of Lung Samples

Group	Sacrifice date [weeks]	Animal No.	Fiber length [µm]				Fiber diameter [µm]			
			Arithmetic		Geometric		Arithmetic		Geometric	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD
All Fibers (L/D > 3)										
Carbon Fiber Low	1	1	11.25	11.41	7.72	2.34	0.97	0.54	0.82	1.80
		2	11.14	10.28	7.99	2.22	0.97	0.47	0.88	1.57
		3	13.19	11.40	9.70	2.17	1.08	0.50	0.98	1.53
		4	12.99	11.84	9.50	2.16	1.05	0.44	0.97	1.51
		5	14.69	13.86	10.45	2.27	1.04	0.51	0.93	1.60
	8	6	11.89	10.52	8.92	2.11	0.91	0.42	0.82	1.59
		7	17.07	15.20	12.75	2.15	1.02	0.49	0.93	1.54
		8	15.48	11.39	12.02	2.07	1.00	0.46	0.90	1.57
		9	14.98	12.16	11.57	2.03	0.97	0.48	0.87	1.59
		10	14.82	12.60	11.03	2.13	1.05	0.48	0.96	1.51
	15	11	17.53	12.62	13.38	2.14	1.01	0.43	0.93	1.50
		12	16.88	14.62	12.21	2.27	0.96	0.48	0.84	1.67
		13	16.03	13.72	11.79	2.18	0.99	0.43	0.90	1.54
		14	17.12	15.23	12.72	2.14	1.00	0.49	0.89	1.67
		15	17.98	13.56	13.65	2.17	1.02	0.50	0.92	1.56
Carbon Fiber Med	1	1	9.50	9.29	7.07	2.08	0.98	0.49	0.88	1.62
		2	11.46	9.91	8.64	2.08	1.01	0.48	0.92	1.57
		3	11.48	10.64	8.30	2.21	0.93	0.44	0.84	1.58
		4	12.62	11.17	9.34	2.15	1.04	0.51	0.93	1.60
		5	9.56	9.05	7.10	2.08	0.99	0.48	0.90	1.58
	8	6	13.96	11.21	10.39	2.21	0.90	0.46	0.79	1.64
		7	14.15	11.38	10.62	2.17	0.92	0.46	0.82	1.61
		8	14.03	9.83	11.22	1.97	1.02	0.50	0.90	1.64
		9	14.69	12.16	11.01	2.15	0.94	0.46	0.84	1.62
		10	14.32	12.74	10.55	2.17	0.96	0.47	0.86	1.62
	15	11	17.79	13.90	13.44	2.16	1.01	0.49	0.91	1.60
		12	14.88	12.15	11.03	2.19	0.98	0.51	0.88	1.58
		13	15.06	13.93	11.06	2.17	1.04	0.50	0.94	1.54
		14	16.33	12.70	12.45	2.11	0.98	0.46	0.88	1.59
		15	16.40	13.68	11.99	2.24	1.01	0.47	0.92	1.56
Carbon Fiber High	1	1	11.96	10.59	8.85	2.16	1.03	0.54	0.91	1.65
		2	12.26	11.54	8.60	2.32	0.97	0.50	0.86	1.65
		3	9.60	9.14	7.03	2.15	0.98	0.53	0.86	1.68
		4	9.52	9.58	6.75	2.23	0.96	0.51	0.84	1.68
		5	13.98	11.57	10.43	2.16	1.03	0.51	0.92	1.61
	8	6	12.69	11.61	9.07	2.26	0.91	0.43	0.82	1.62
		7	13.87	14.36	9.70	2.27	0.96	0.49	0.85	1.64
		8	13.88	12.84	9.86	2.27	0.94	0.48	0.83	1.66
		9	14.94	10.84	11.46	2.14	0.97	0.48	0.86	1.63
		10	13.51	13.10	9.48	2.29	0.95	0.47	0.84	1.63
	15	11	17.09	14.75	13.05	2.08	1.09	0.59	0.96	1.66
		12	16.86	14.25	12.68	2.12	1.05	0.54	0.94	1.62
		13	15.65	12.54	11.97	2.08	1.02	0.49	0.92	1.58
		14	15.06	12.31	11.19	2.18	1.04	0.53	0.92	1.65
		15	15.21	13.50	11.34	2.13	1.02	0.49	0.92	1.58

SD: Standard deviation

Appendix 5b (cont.) Fiber Size Distribution of Lung Samples

Group	Sacrifice date [weeks]	Animal No.	Fiber length [µm]				Fiber diameter [µm]			
			Arithmetic		Geometric		Arithmetic		Geometric	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD
Fibers L > 20										
Carbon Fiber Low	1	1	32.91	13.37	30.91	1.40	1.11	0.54	1.00	1.58
		2	30.78	10.21	29.50	1.32	1.10	0.49	1.01	1.51
		3	32.40	11.24	30.78	1.36	1.25	0.49	1.16	1.48
		4	32.37	13.00	30.45	1.39	1.14	0.47	1.06	1.49
		5	34.72	15.73	32.28	1.43	1.12	0.46	1.03	1.52
	8	6	32.77	12.83	30.89	1.38	1.04	0.37	0.98	1.43
		7	34.74	17.94	32.12	1.43	1.13	0.60	1.02	1.55
		8	30.64	10.26	29.23	1.34	1.02	0.39	0.95	1.47
		9	32.40	12.82	30.45	1.40	1.03	0.49	0.95	1.48
		10	33.02	12.39	31.20	1.38	1.22	0.61	1.09	1.60
	15	11	32.29	9.42	31.01	1.33	1.06	0.42	0.98	1.48
		12	35.11	14.73	32.67	1.44	1.02	0.51	0.91	1.60
		13	35.00	13.06	33.04	1.39	1.07	0.42	0.99	1.50
		14	34.70	17.05	32.05	1.44	1.18	0.52	1.08	1.52
		15	33.09	12.28	31.26	1.38	1.18	0.59	1.07	1.55
Carbon Fiber Med	1	1	31.81	14.51	29.69	1.41	1.21	0.64	1.10	1.52
		2	32.31	11.20	30.76	1.35	1.09	0.48	1.01	1.50
		3	31.87	12.00	30.32	1.34	1.01	0.43	0.94	1.47
		4	33.41	12.95	31.49	1.39	1.12	0.56	1.00	1.57
		5	31.98	11.91	30.31	1.36	1.20	0.48	1.10	1.51
	8	6	31.02	11.05	29.46	1.36	0.99	0.44	0.90	1.55
		7	32.64	11.46	30.97	1.37	1.08	0.59	0.96	1.60
		8	28.57	8.76	27.54	1.29	1.12	0.58	0.98	1.69
		9	33.19	13.02	31.18	1.40	1.08	0.46	0.99	1.52
		10	33.71	14.73	31.27	1.44	0.97	0.43	0.88	1.55
	15	11	34.44	13.08	32.46	1.39	1.11	0.44	1.02	1.54
		12	33.00	10.76	31.51	1.34	1.13	0.49	1.05	1.49
		13	33.58	16.56	31.21	1.42	1.25	0.61	1.13	1.57
		14	33.15	11.74	31.37	1.38	0.98	0.43	0.90	1.50
		15	34.18	13.35	32.00	1.42	1.13	0.42	1.06	1.45
Carbon Fiber High	1	1	32.11	12.62	30.32	1.37	1.13	0.62	1.00	1.62
		2	32.09	13.14	30.11	1.40	1.08	0.51	0.97	1.58
		3	30.53	12.34	28.83	1.37	1.27	0.56	1.16	1.55
		4	31.45	11.76	29.79	1.37	1.11	0.55	0.99	1.62
		5	31.02	11.66	29.32	1.38	1.11	0.52	1.01	1.56
	8	6	32.27	12.19	30.47	1.38	1.02	0.47	0.92	1.58
		7	36.50	19.01	33.12	1.51	1.08	0.55	0.97	1.58
		8	33.74	13.83	31.60	1.41	1.04	0.59	0.92	1.64
		9	30.51	9.79	29.20	1.33	1.11	0.48	1.01	1.54
		10	34.05	14.93	31.69	1.43	1.03	0.51	0.92	1.59
	15	11	33.49	17.49	30.96	1.43	1.27	0.70	1.09	1.74
		12	35.17	15.42	32.80	1.42	1.16	0.58	1.04	1.59
		13	32.65	12.65	30.78	1.39	1.09	0.52	0.99	1.57
		14	32.66	11.49	31.00	1.36	1.15	0.57	1.03	1.61
		15	33.02	15.80	30.68	1.42	1.08	0.43	1.00	1.49

SD: Standard deviation

Appendix 5b (cont.) Fiber Size Distribution of Lung Samples

Group	Sacrifice date [weeks]	Animal No.	Fiber length [µm]				Fiber diameter [µm]			
			Arithmetic		Geometric		Arithmetic		Geometric	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD
WHO Fibers										
Carbon Fiber Low	1	1	15.00	12.12	11.95	1.89	1.13	0.51	1.01	1.62
		2	14.57	10.70	11.82	1.86	1.10	0.49	1.00	1.55
		3	16.09	11.61	13.11	1.85	1.13	0.45	1.05	1.49
		4	15.65	12.17	12.55	1.88	1.14	0.45	1.06	1.47
		5	17.60	14.24	13.93	1.92	1.12	0.48	1.02	1.54
	8	6	14.17	10.75	11.68	1.79	0.98	0.43	0.89	1.56
		7	18.70	15.04	15.05	1.88	1.04	0.43	0.96	1.51
		8	17.12	11.23	14.25	1.81	1.03	0.43	0.94	1.52
		9	16.39	12.22	13.39	1.83	0.98	0.44	0.89	1.55
		10	16.64	12.72	13.23	1.93	1.08	0.46	0.99	1.49
	15	11	19.39	12.33	15.89	1.89	1.04	0.42	0.97	1.49
		12	19.28	14.64	15.34	1.93	1.02	0.49	0.91	1.63
		13	18.14	13.83	14.34	1.95	1.04	0.43	0.96	1.51
		14	18.68	15.34	14.75	1.93	1.05	0.49	0.94	1.63
		15	19.48	13.34	15.89	1.88	1.03	0.44	0.95	1.51
Carbon Fiber Med	1	1	12.48	10.01	10.38	1.74	1.12	0.48	1.02	1.56
		2	14.06	10.20	11.63	1.79	1.09	0.48	1.00	1.53
		3	14.43	10.99	11.75	1.83	1.03	0.44	0.95	1.52
		4	15.29	11.42	12.57	1.81	1.12	0.47	1.03	1.51
		5	13.25	9.84	11.07	1.75	1.15	0.47	1.06	1.52
	8	6	16.68	11.05	13.97	1.79	0.97	0.46	0.87	1.59
		7	16.51	11.26	13.74	1.80	0.97	0.44	0.88	1.56
		8	15.54	9.65	13.20	1.75	1.06	0.49	0.96	1.59
		9	16.88	12.17	13.82	1.85	1.00	0.47	0.90	1.60
		10	16.75	12.96	13.52	1.86	1.00	0.45	0.90	1.59
	15	11	19.55	13.79	15.86	1.89	1.05	0.45	0.96	1.55
		12	17.28	12.14	14.02	1.88	1.04	0.45	0.95	1.52
		13	17.24	14.26	13.66	1.92	1.08	0.48	0.98	1.53
		14	17.69	12.60	14.31	1.89	1.01	0.46	0.92	1.56
		15	18.80	13.63	15.14	1.90	1.07	0.44	0.99	1.50
Carbon Fiber High	1	1	14.63	10.83	12.08	1.80	1.13	0.49	1.02	1.56
		2	15.98	11.91	13.01	1.85	1.07	0.49	0.97	1.58
		3	13.03	9.81	10.87	1.75	1.13	0.53	1.01	1.60
		4	13.28	10.48	10.80	1.81	1.09	0.48	0.99	1.59
		5	16.41	11.58	13.49	1.83	1.11	0.51	1.00	1.56
	8	6	15.59	11.89	12.53	1.88	1.00	0.43	0.91	1.56
		7	16.83	15.00	13.13	1.93	1.05	0.49	0.94	1.60
		8	16.83	13.15	13.39	1.92	1.01	0.45	0.91	1.61
		9	17.31	10.48	14.74	1.76	1.03	0.44	0.93	1.56
		10	16.40	13.52	12.90	1.93	1.02	0.47	0.92	1.60
	15	11	18.62	14.87	15.10	1.86	1.12	0.54	0.99	1.63
		12	18.18	14.31	14.44	1.93	1.07	0.50	0.96	1.60
		13	17.21	12.54	14.01	1.86	1.06	0.47	0.97	1.54
		14	17.08	11.93	13.92	1.87	1.10	0.51	0.99	1.61
		15	17.17	13.65	13.80	1.88	1.08	0.49	0.98	1.56

SD: Standard deviation

Appendix 6 Validation of Lung Tissue Dissolving Procedure

Sample		Concentration per µg test fibers in sample							
		Fibers	Fibers L<=5µm	Fibers L=5-20µm	WHO Fibers	Fibers L>20µm	Particles	Estimated mass [µg]*	
								Fibers	Particles
Inst. Suspension with treatment	50µg	30745	7469	18687	23276	4589	10621	0.753	0.062
	100µg	31135	6074	18811	25062	6251	9907	0.879	0.077
	500µg	30110	5304	19247	24806	5559	6987	0.856	0.046
	Mean	30664	6282	18915	24381	5466	9172	0.829	0.062
	SD	517	1098	294	966	835	1925	0.067	0.015
Lung 0.05mg	2	29001	4128	18505	24873	6369	8904	1.078	0.106
	3	25364	4199	15882	21166	5284	7312	0.708	0.061
	Mean	27183	4163	17193	23020	5826	8108	0.893	0.083
	SD	2572	50	1854	2622	767	1126	0.262	0.032
Lung 0.1mg	1	33903	6982	19876	26921	7045	10630	0.940	0.046
	2	27028	4162	16983	22866	5883	5217	0.861	0.049
	3	29487	5716	17555	23771	6216	8769	0.803	0.068
	Mean	30140	5620	18138	24519	6381	8205	0.868	0.055
	SD	3483	1412	1532	2129	598	2750	0.069	0.012
Lung 0.5mg	1	30470	6254	18069	24216	6146	7871	0.934	0.070
	2	31357	7714	17649	23643	5994	7215	0.700	0.082
	3	36404	8547	22085	27857	5772	8269	0.966	0.144
	Mean	32744	7505	19268	25238	5971	7785	0.867	0.099
	SD	3201	1161	2449	2286	188	533	0.145	0.040
Mean		30455	6050	18486	24405	5919	8337	0.862	0.074
SD		2991	1526	1625	1850	636	1659	0.116	0.029
Inst. Suspension without treatment	50µg	36459	9534	20497	26924	6428	11558	0.764	0.046
	100µg	40212	10378	22789	29834	7045	11551	0.859	0.117
	500µg	37879	10656	21161	27223	6061	10878	0.773	0.057
	Mean	38183	10190	21482	27994	6511	11329	0.799	0.074
	SD	1895	584	1179	1601	497	390	0.052	0.038

SD: Standard deviation

* Definition of fibers and particles and method for estimation of mass see **3.4.3**

Appendix 6 (cont.) Validation of Lung Tissue Dissolving Procedure

Size distribution of fibers in dissolved lung and suspension samples

Sample		Fiber length [µm]				Fiber diameter [µm]			
		Arithmetic		Geometric		Arithmetic		Geometric	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Fraction L/D>3									
Inst. Suspension with treatment	50µg	1281	1425	887	226	1.14	0.64	99	169
	100µg	1490	1526	10.45	226	118	59	105	164
	500µg	14.26	16.33	9.98	2.24	1.17	0.68	1.01	172
	Mean	13.99	15.28	9.77	2.25	1.16	0.64	1.02	1.68
	S.D.	0.91	0.89	0.69	0.01	0.02	0.04	0.02	0.03
Lung 0.05mg	2	15.85	18.37	11.24	2.19	1.28	0.70	1.12	1.65
	3	14.86	14.45	10.78	2.18	1.24	0.65	1.09	1.67
Lung 0.1mg	1	13.62	13.93	9.58	2.26	1.21	0.68	1.05	1.73
	2	17.17	23.26	11.47	2.30	1.24	0.60	1.11	1.61
	3	15.14	19.06	10.24	2.29	1.20	0.65	1.05	1.69
Lung 0.5mg	1	14.69	17.17	9.85	2.30	1.14	0.61	1.01	1.63
	2	13.07	13.30	9.07	2.28	1.09	0.56	0.97	1.62
	3	13.97	19.76	9.26	2.27	1.12	0.60	0.98	1.66
Lungs	Mean	14.80	17.41	10.19	2.26	1.19	0.63	1.05	1.66
	SD	1.24	3.24	0.86	0.05	0.06	0.04	0.05	0.04
Inst. Suspension without treatment	50µg	13.65	17.30	9.06	2.37	1.09	0.62	0.93	1.75
	100µg	12.63	13.16	8.94	2.23	1.13	0.61	0.99	1.69
	500µg	12.87	14.81	8.70	2.30	1.03	0.60	0.88	1.75
	Mean	13.05	15.09	8.90	2.30	1.08	0.61	0.94	1.73
	SD	0.46	1.78	0.16	0.06	0.04	0.01	0.04	0.03

SD: Standard deviation

Appendix 6 (cont.) Validation of Lung Tissue Dissolving Procedure

Size distribution of fibers in dissolved lung and suspension samples

Sample		Fiber length [µm]				Fiber diameter [µm]			
		Arithmetic		Geometric		Arithmetic		Geometric	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Fraction L>20µm									
Inst. Suspension with treatment	50µg	39.31	20.54	35.53	1.53	1.46	0.83	1.26	1.71
	100µg	38.49	19.35	34.90	1.52	1.57	0.78	1.38	1.69
	500µg	38.46	25.31	33.89	1.57	1.48	0.82	1.30	166
	Mean	38.75	21.73	34.77	1.54	1.50	0.81	1.31	1.69
	SD	0.41	2.69	0.71	0.02	0.05	0.02	0.05	0.02
Lung 0.05mg	2	38.88	28.00	34.03	1.58	1.48	0.79	1.30	1.68
	3	36.32	18.49	33.23	1.48	1.49	0.80	1.33	1.59
Lung 0.1mg	1	34.28	18.00	31.24	1.49	1.60	0.77	1.44	1.58
	2	44.82	37.82	37.50	1.69	1.41	0.71	1.26	1.61
	3	39.15	30.15	33.48	1.64	1.45	0.75	1.27	1.67
Lung 0.5mg	1	40.58	23.52	35.83	1.60	1.54	0.82	1.35	167
	2	35.44	15.18	32.93	1.44	1.40	0.65	1.26	1.58
	3	44.15	35.70	37.11	1.69	1.49	0.72	1.32	1.66
Lungs	Mean	39.20	25.86	34.42	1.57	1.48	0.75	1.32	1.63
	SD	3.68	8.02	2.08	0.09	0.06	0.05	0.06	0
Inst. Suspension without treatment	50µg	39.16	28.45	34.01	1.60	1.26	0.65	1.11	1.68
	100µg	33.92	18.55	30.76	1.50	1.45	0.70	1.30	1.61
	500µg	38.89	21.34	34.87	1.55	1.38	0.67	1.23	1.64
	Mean	37.32	22.78	33.21	1.55	1.36	0.67	1.21	1.65
	SD	2.51	4.35	1.85	0.04	0.08	0.02	0.08	0

SD: Standard deviation

Appendix 6 (cont.) Validation of Lung Tissue Dissolving Procedure

Size distribution of fibers in dissolved lung and suspension samples

Sample		Fiber length [µm]				Fiber diameter [µm]			
		Arithmetic		Geometric		Arithmetic		Geometric	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Fraction WHO fibers									
Inst. Suspension with treatment	50µg	15.84	15.17	12.22	1.92	1.29	0.66	1.14	1.64
	100µg	17.60	15.87	13.51	1.97	1.28	0.60	1.15	1.59
	500µg	16.59	17.11	12.75	1.92	1.27	0.69	1.12	1.64
	Mean	16.67	16.05	12.83	1.94	1.28	0.65	1.13	1.63
	SD	0.75	0.84	0.55	0.03	0.01	0.04	0.01	0.02
Lung 0.05mg	2	17.88	19.09	13.60	1.96	1.36	0.71	1.21	1.63
	3	17.10	14.83	13.50	1.90	1.34	0.65	1.21	1.57
Lung 0.1mg	1	16.30	14.47	12.76	1.91	1.34	0.69	1.18	1.68
	2	19.63	24.50	14.21	2.04	1.31	0.61	1.19	1.57
	3	17.91	20.26	13.26	2.01	1.32	0.66	1.18	1.62
Lung 0.5mg	1	17.55	18.20	12.84	2.05	1.24	0.64	1.11	1.62
	2	16.18	13.95	12.50	1.96	1.21	0.59	1.08	1.60
	3	17.15	21.61	12.48	2.00	1.24	0.62	1.10	1.64
Lungs	Mean	17.46	18.36	13.14	1.98	1.30	0.64	1.16	1.62
	SD	1.03	3.58	0.57	0.05	0.06	0.04	0.05	0.03
Inst. Suspension without treatment	50µg	17.32	18.80	13.22	1.93	1.24	0.64	1.09	1.68
	100µg	15.80	13.93	12.55	1.87	1.28	0.63	1.13	1.65
	500µg	16.51	16.05	12.53	1.97	1.17	0.62	1.03	1.69
	Mean	16.55	16.26	12.77	1.92	1.23	0.63	1.08	1.67
	SD	0.65	2.08	0.33	0.04	0.04	0.01	0.05	0.02

SD: Standard deviation

Appendix 7 Retention of Fibers in the Lungs

Sacrifice [weeks]	An. No.	Number of Fibers [10^6/lung]					Particles [10^6/lung]	Estimated mass [µg/lung]*	
		All	L≤5µm	L=5-20µm	WHO Fibers	L> 20µm		Fibers	Particles
Control									
1 Week	1	0.0	0.0	0.0	0.0	0.002	0.0	0	0
	2	0.0	0.0	0.0	0.0	0.000	0.0	0	0
	3	0.0	0.0	0.0	0.0	0.000	0.0	0	0
	5	0.0	0.0	0.0	0.0	0.000	0.0	0	0
	31	0.0	0.0	0.0	0.0	0.000	0.0	0	0
8 Weeks	6	0.0	0.0	0.0	0.0	0.000	0.0	0	0
	7	0.0	0.0	0.0	0.0	0.000	0.0	0	0
	8	0.0	0.0	0.0	0.0	0.000	0.0	0	0
	9	0.0	0.0	0.0	0.0	0.000	0.0	0	0
	10	0.0	0.0	0.0	0.0	0.000	0.0	0	0
15 Weeks	11	0.0	0.0	0.0	0.0	0.000	0.0	0	0
	12	0.0	0.0	0.0	0.0	0.000	0.0	0	0
	13	0.0	0.0	0.0	0.0	0.000	0.0	0	0
	14	0.0	0.0	0.0	0.0	0.000	0.0	0	0
	15	0.0	0.0	0.0	0.0	0.000	0.0	0	0
Carbon fiber low									
1 Week	1	18.4	5.9	9.7	12.5	2.835	4.5	250	29
	2	24.0	7.5	12.8	16.6	3.803	8.1	290	41
	3	15.5	3.6	8.9	11.9	2.992	5.4	270	22
	4	15.4	3.4	8.9	11.9	3.000	4.9	231	26
	5	16.3	3.4	9.1	12.8	3.708	4.9	293	21
8 Weeks	6	18.3	3.9	11.9	14.5	2.536	21.7	199	47
	7	11.9	1.4	7.1	10.5	3.381	2.9	240	8
	8	13.0	1.6	7.9	11.4	3.501	4.1	202	28
	9	13.0	1.4	8.4	11.6	3.122	3.6	193	15
	10	11.7	1.7	7.1	10.0	2.855	3.2	216	10
15 Weeks	11	8.3	1.0	4.5	7.3	2.857	2.3	146	11
	12	7.9	1.2	4.4	6.7	2.293	2.2	130	9
	13	8.5	1.3	5.0	7.2	2.235	2.3	140	9
	14	8.2	0.9	4.9	7.4	2.466	2.8	159	9
	15	8.0	0.8	4.4	7.2	2.709	2.6	169	10

* Definition of fibers and particles and method for estimation of mass see **3.4.3**

Appendix 7 (cont.) Retention of Fibers in the Lungs

Sacrifice [weeks]	An. No.	Number of Fibers [10^6/lung]					Particles [10^6/lung]	Estimated mass [µg/lung]*	
		All	L≤5µm	L=5-20µm	WHO Fibers	L> 20µm		Fibers	Particles
Carbon fiber med									
1 Week	1	96.5	32.0	55.6	64.5	8.891	40.7	1061	218
	2	91.0	22.8	56.6	68.2	11.636	30.3	1197	154
	3	85.7	22.6	50.1	63.1	12.973	36.1	943	146
	4	76.7	17.1	47.7	59.6	11.949	23.5	1150	106
	5	88.2	33.7	46.1	54.4	8.339	29.2	995	146
8 Weeks	6	55.6	11.4	32.3	44.2	11.956	29.0	694	103
	7	46.8	8.8	28.8	38.0	9.174	13.5	665	52
	8	47.2	6.4	30.0	40.9	10.902	11.8	766	63
	9	46.2	7.6	28.7	38.5	9.860	13.2	666	51
	10	48.6	8.8	29.6	39.8	10.163	18.1	645	66
15 Weeks	11	31.2	3.4	18.1	27.8	9.645	7.8	610	59
	12	37.0	6.5	21.7	30.5	8.821	12.3	616	39
	13	40.0	6.4	23.8	33.6	9.780	10.7	776	68
	14	33.9	3.2	21.3	30.7	9.361	8.2	534	42
	15	36.5	5.7	20.7	30.8	10.090	9.7	670	74
Carbon fiber high									
1 Week	1	200.3	48.4	122.7	151.9	29.186	80.8	3076	457
	2	183.2	54.2	97.2	129.0	31.878	52.7	2496	302
	3	180.3	63.8	97.5	116.5	18.962	63.5	2271	349
	4	243.5	91.4	124.6	152.1	27.465	79.0	2708	275
	5	218.2	41.5	126.7	176.7	49.998	79.5	3525	373
8 Weeks	6	144.0	34.7	82.1	109.3	27.193	57.4	1679	150
	7	140.3	31.9	81.8	108.3	26.568	50.0	2086	132
	8	131.4	29.7	73.8	101.7	27.905	44.3	1839	201
	9	139.9	24.1	83.2	115.8	32.600	34.4	2254	203
	10	131.2	29.5	74.7	101.7	27.055	42.1	1788	144
15 Weeks	11	109.1	11.3	65.5	97.7	32.230	29.9	2654	133
	12	100.9	9.3	64.5	91.6	27.095	40.4	2040	349
	13	115.2	13.2	72.1	102.0	29.921	34.1	1964	121
	14	115.1	18.6	68.0	96.4	28.424	26.4	2161	113
	15	118.7	17.1	72.1	101.6	29.468	25.7	1929	192

* Definition of fibers and particles and method for estimation of mass see **3.4.3**

Appendix 8 Histopathological Investigation of Individual Animals

FRAUNHOFER ITA

02G02022

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Unmerged Histopathological Findings, 5-days postexposure subgroup

LESIONS	TREATMENT	INCIDENCE OF LESIONS (ANIMA! Males			
		Clean Air	~15 F/ml	~50 F/ml	~150 F/ml
TRACHEA		(5)	(5)	(4)	(5)
No abnormality detected		010001 010002 010003 010005 010031	020001 020002 020003 020004 020005	030002 030004 030005	040001 040002 040003 040004
Multifocal mucous (goblet) cell hyperplasia very slight					040005
Score Expanded Totals					1
Focal mucous (goblet) cell hyperplasia very slight				030003	
Score Expanded Totals				1	
LUNGS		(5)	(5)	(5)	(5)
EPS grade 2				030003 030004	040001 040002 040003 040004 040005
EPS grade 1			020001 020002 020003 020004 020005	030001 030002 030005	
EPS grade 0		010001 010002 010003 010005 010031			
Wagner grade 4 : 0,80%				030003	
Wagner grade 4 : 0,67%				030004	
Wagner grade 4 : 1,27%					040001
Wagner grade 4 : 1,85%					040002
Wagner grade 4 : 1,59%					040003
Wagner grade 4 : 1,81%					040004
Wagner grade 4 : 1,54%					040005
Wagner grade 3			020001 020002	030001 030002	

Figures in brackets represent the number of animals from which this tissue was examined microscopically
The absence of a numeral indicates that the lesion specified was not identified

*** Continued

Appendix 8 (cont.) Histopathological Investigation of Individual Animals

FRAUNHOFER ITA

02G02022

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Unmerged Histopathological Findings, 5-days postexposure subgroup

LESIONS	TREATMENT	INCIDENCE OF LESIONS (ANIMA! Males
		Clean Air	~15 F/ml	~50 F/ml	~150 F/ml
LUNGS					
Wagner grade 3		(5)	(5)	(5)	(5)
			020003	030005	
Wagner grade 1			020004		
			020005		
		010001			
		010002			
		010003			
		010005			
Multifocal microgranuloma(s)		010031			
very slight					
			020001		
			020002		
			020003		
			020004		
slight			020005		
				030001	040001
				030002	040002
				030003	040003
moderate				030004	
				030005	
Score Expanded Totals					040004
					040005
Multifocal accumulation of fibre-laden macrophages			5	5	5
very slight					
			020001		
			020002		
			020003		
			020004		
slight			020005		
				030001	040001
				030002	
				030003	
				030004	
moderate				030005	
					040002
					040003
					040004
Score Expanded Totals					040005
			5	5	5

Figures in brackets represent the number of animals from which this tissue was examined microscopically
The absence of a numeral indicates that the lesion specified was not identified

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Appendix 8 (cont.) Histopathological Investigation of Individual Animals

FRAUNHOFER ITA

02G02022

Page: 3
16-DEC-02

Unmerged Histopathological Findings, 5-days postexposure subgroup

LESIONS	TREATMENT	INCIDENCE OF LESIONS (ANIMA! Males			
		Clean Air	~15 F/ml	~50 F/ml	~150 F/ml
LUNGS		(5)	(5)	(5)	(5)
Multifocal bronchiolo-alveolar hyperplasia					
very slight			020001		
			020002		
			020004		
slight			020005		
			020003	030001	040001
				030002	040002
				030003	040003
				030004	040004
Score Expanded Totals				030005	040005
Multifocal interstitial fibrosis		5	5	5	5
very slight			020001	030001	
			020002	030002	
			020003	030005	
			020004		
slight			020005		
				030003	040001
				030004	040002
					040003
					040004
Score Expanded Totals					040005
Multifocal interstitial multinucleate giant-cell(s)		5	5	5	5
very slight					
slight				030004	040001
					040002
					040003
					040004
Score Expanded Totals					040005
Focal interstitial multinucleate giant-cell(s)				1	5
very slight					
Score Expanded Totals				030005	
Multifocal bronchiolar mucous (goblet) cell hyperplasia				1	
very slight					
				030004	040001
slight					040002
					040003
					040004

Figures in brackets represent the number of animals from which this tissue was examined microscopically
The absence of a numeral indicates that the lesion specified was not identified

*** Continued

Appendix 8 (cont.) Histopathological Investigation of Individual Animals

FRAUNHOFER ITA

02G02022

Page: 4
16-DEC-02

Unmerged Histopathological Findings, 5-days postexposure subgroup

LESIONS	TREATMENT	INCIDENCE OF LESIONS (ANIMA! Males			
		Clean Air	~15 F/ml	~50 F/ml	~150 F/ml
LUNGS		(5)	(5)	(5)	(5)
Multifocal bronchiolar mucous (goblet) cell hyperplasia					
slight					
Score Expanded Totals					040005
Focal bronchiolar mucous (goblet) cell hyperplasia				1	5
slight					
Score Expanded Totals				030001	
Focal alveolar histiocytosis				1	
very slight					
Score Expanded Totals		010002			
		1			

Figures in brackets represent the number of animals from which this tissue was examined microscopically
The absence of a numeral indicates that the lesion specified was not identified

*** Listing Complete ***

Appendix 8 (cont.) Histopathological Investigation of Individual Animals

FRAUNHOFER ITA 02G02022 Page: 1
21-MAR-03

Unmerged Histopathological Findings, 8 weeks postexposure subgroup

LESIONS	TREATMENT	INCIDENCE OF LESIONS (ANIMA! Males			
		Clean Air	15 F/ml	50 F/ml	150 F/ml
		(5)	(5)	(5)	(5)
TRACHEA					
No abnormality detected		010006 010007 010008 010009 010010	020006 020007 020008 020009 020010	030006 030007 030008 030009 030010	040006 040007 040008 040009 040010
		(5)	(5)	(5)	(5)
LUNGS					
EPS grade 2				030010	040006 040007 040008 040009 040010
EPS grade 1			020006 020007 020008 020009 020010	030006 030007 030008 030009	
EPS grade 0		010006 010007 010008 010009 010010			
Wagner grade 4 : 0,63%					040010
Wagner grade 4 : 0,68%				030010	
Wagner grade 4 : 0,81%					040007
Wagner grade 4 : 0,91%					040008
Wagner grade 4 : 0,99%					040006
Wagner grade 4 : 1,10%					040009
Wagner grade 3			020006 020007 020008 020009 020010	030006 030007 030008 030009	
Wagner grade 1		010006 010007 010008 010009			

Figures in brackets represent the number of animals from which this tissue was examined microscopically
The absence of a numeral indicates that the lesion specified was not identified

*** Continued

Appendix 8 (cont.) Histopathological Investigation of Individual Animals

FRAUNHOFER ITA 02G02022 Page: 2
21-MAR-03

Unmerged Histopathological Findings, 8 weeks postexposure subgroup

LESIONS	TREATMENT	INCIDENCE OF LESIONS (ANIMA! Males			
		Clean Air	~15 F/ml	~50 F/ml	~150 F/ml
LUNGS		(5)	(5)	(5)	(5)
Wagner grade 1		010010			
Multifocal accumulation of fibre-laden macrophages					
very slight			020006		
			020007		
			020008		
			020010		
slight			020009	030006	040007
				030007	040010
				030008	
				030009	
				030010	
moderate					040006
					040008
					040009
Score Expanded Totals			5	5	5
Multifocal microgranuloma(s)					
very slight			020006		
			020007		
			020008		
			020010		
slight			020009	030006	040007
				030007	040009
				030008	040010
				030009	
				030010	
moderate					040006
					040008
Score Expanded Totals			5	5	5
Multifocal interstitial multinucleate giant-cell(s)					
very slight			020009	030006	
				030007	
				030008	
				030009	
				030010	
slight					040006
					040007
					040008
					040009
					040010

Figures in brackets represent the number of animals from which this tissue was examined microscopically
The absence of a numeral indicates that the lesion specified was not identified

*** Continued

Appendix 8 (cont.) Histopathological Investigation of Individual Animals

FRAUNHOFER ITA 02G02022 Page: 3
21-MAR-03

Unmerged Histopathological Findings, 8 weeks postexposure subgroup

LESIONS	TREATMENT	INCIDENCE OF LESIONS (ANIMA			
		Males			
		Clean Air	~15 F/ml	~50 F/ml	~150 F/ml
LUNGS		(5)	(5)	(5)	(5)
Multifocal interstitial multinucleate giant-cell(s)					
Score Expanded Totals			1	5	5
Multifocal interstitial fibrosis					
very slight			020006	030006	
			020007	030007	
			020008	030008	
			020009	030009	
			020010		
slight				030010	040006
					040007
					040008
					040009
					040010
Score Expanded Totals			5	5	5
Multifocal bronchiolo-alveolar hyperplasia					
very slight			020007		
			020008		
			020010		
slight			020009	030006	040006
				030007	040007
				030008	040008
				030009	040009
				030010	040010
Score Expanded Totals			4	5	5
Focal bronchiolo-alveolar hyperplasia					
slight			020006		
Score Expanded Totals			1		
Multifocal bronchiolar mucous (goblet) cell hyperplasia					
very slight					040007
					040008
slight					040006
Score Expanded Totals					3
Focal bronchiolar mucous (goblet) cell hyperplasia					
very slight					040010
Score Expanded Totals					1
Multifocal mucous (goblet) cell metaplasia					
very slight				030009	040008
slight				030006	040006

Figures in brackets represent the number of animals from which this tissue was examined microscopically
The absence of a numeral indicates that the lesion specified was not identified

*** Continued

Appendix 8 (cont.) Histopathological Investigation of Individual Animals

FRAUNHOFER ITA 02G02022 Page: 4
21-MAR-03

Unmerged Histopathological Findings, 8 weeks postexposure subgroup

LESIONS	TREATMENT	INCIDENCE OF LESIONS (ANIMA			
		Males			
		Clean Air	~15 F/ml	~50 F/ml	~150 F/ml
LUNGS		(5)	(5)	(5)	(5)
Multifocal mucous (goblet) cell metaplasia					
Score Expanded Totals				2	2
Focal mucous (goblet) cell metaplasia					
very slight				04	00 10
slight				04	00 09
Score Expanded Totals					2
Focal alveolar inflammatory cell infiltration					
slight			02	00 10	
Score Expanded Totals			1		

Figures in brackets represent the number of animals from which this tissue was examined microscopically
The absence of a numeral indicates that the lesion specified was not identified

*** Listing Complete ***

Appendix 8 (cont.) Histopathological Investigation of Individual Animals

FRAUNHOFER ITA 02G02022 Page: 1
21-MAR-03

Unmerged Histopathological Findings, 15 weeks postexposure subgroup

LESIONS	TREATMENT	INCIDENCE OF LESIONS (ANIMA				
		Males				
		Clean Air	~15 F/ml	~50 F/ml	~150 F/ml	
TRACHEA		(5)	(5)	(5)	(5)	
No abnormality detected		010011	020011	030011	040011	
		010012	020012	030012	040012	
		010013	020013	030014	040013	
		010014	020014	030015	040014	
		010015	020015		040015	
Multifocal mucous (goblet) cell hyperplasia						
slight				030013		
Score Expanded Totals				1		
LUNGS		(5)	(5)	(5)	(5)	
EPS grade 2				030012	040011	
					040012	
					040013	
					040014	
					040015	
EPS grade 1			020011	030011		
			020012	030013		
			020013	030014		
			020014	030015		
			020015			
EPS grade 0		010011				
		010012				
		010013				
		010014				
		010015				
Wagner grade 4 : 0,90%					040015	
Wagner grade 4 : 0,93%				030012		
Wagner grade 4 : 1,00%					040013	
Wagner grade 4 : 1,13%					040012	
Wagner grade 4 : 1,15%					040011	
Wagner grade 4 : 1,24%					040014	
Wagner grade 3			020011	030011		
			020012	030013		
			020013	030014		
			020014	030015		
			020015			
Wagner grade 1		010011				

Figures in brackets represent the number of animals from which this tissue was examined microscopically
The absence of a numeral indicates that the lesion specified was not identified

*** Continued

Appendix 8 (cont.) Histopathological Investigation of Individual Animals

FRAUNHOFER ITA 02G02022 Page: 2
21-MAR-03

Unmerged Histopathological Findings, 15 weeks postexposure subgroup

LESIONS	TREATMENT	INCIDENCE OF LESIONS (ANIMA! Males			
		Clean Air	~15 F/ml	~50 F/ml	~150 F/ml
		(5)	(5)	(5)	(5)
LUNGS					
Wagner grade 1		010012			
		010013			
		010014			
		010015			
Multifocal accumulation of fibre-laden macrophages					
very slight			020011		
			020012		
			020013		
			020014		
			020015		
slight				030011	040011
				030012	
				030013	
				030014	
				030015	
moderate					040012
					040013
					040014
					040015
Score Expanded Totals			5	5	5
Multifocal microgranuloma(s)					
very slight			020011		
			020012		
			020013		
			020014		
			020015		
slight				030011	040011
				030012	040014
				030013	040015
				030014	
				030015	
moderate					040012
					040013
Score Expanded Totals			5	5	5
Multifocal interstitial multinucleate giant-cell(s)					
very slight			020014	030011	
				030014	
				030015	
slight				030012	040011

Figures in brackets represent the number of animals from which this tissue was examined microscopically
The absence of a numeral indicates that the lesion specified was not identified

*** Continued

Appendix 8 (cont.) Histopathological Investigation of Individual Animals

FRAUNHOFER ITA 02G02022 Page: 3
21-MAR-03

Unmerged Histopathological Findings, 15 weeks postexposure subgroup

LESIONS	TREATMENT	INCIDENCE OF LESIONS (ANIMA			
		Males			
		Clean Air	~15 F/ml	~50 F/ml	~150 F/ml
LUNGS		(5)	(5)	(5)	(5)
Multifocal interstitial multinucleate giant-cell(s)					
slight				030013	040012
					040013
					040014
					040015
Score Expanded Totals			1	5	5
Multifocal interstitial fibrosis					
very slight			020012	030011	
			020014	030013	
			020015	030014	
				030015	
slight				030012	040011
					040012
					040013
					040014
					040015
Score Expanded Totals			3	5	5
Multifocal bronchiolo-alveolar hyperplasia					
very slight			020011	030014	
			020014		
			020015		
slight				030011	040011
				030012	040012
				030013	040013
				030015	040014
					040015
Score Expanded Totals			3	5	5
Focal bronchiolo-alveolar hyperplasia					
slight			020012		
Score Expanded Totals			1		
Multifocal bronchiolar mucous (goblet) cell hyperplasia					
very slight				030012	040011
					040014
slight				030013	
Score Expanded Totals				2	2
Multifocal mucous (goblet) cell metaplasia					
very slight					040011
					040012

Figures in brackets represent the number of animals from which this tissue was examined microscopically
The absence of a numeral indicates that the lesion specified was not identified

*** Continued

Appendix 8 (cont.) Histopathological Investigation of Individual Animals

FRAUNHOFER ITA 02G02022 Page: 4
21-MAR-03

Unmerged Histopathological Findings, 15 weeks postexposure subgroup

LESIONS	TREATMENT	INCIDENCE OF LESIONS (ANIMA				
		Males				
		Clean Air	~15 F/ml	~50 F/ml	~150 F/ml	
LUNGS		(5)	(5)	(5)	(5)	
Multifocal mucous (goblet) cell metaplasia						
very slight					040014	
slight					040013	
Score Expanded Totals					4	
Focal mucous (goblet) cell metaplasia						
very slight				030014		
				030015		
Score Expanded Totals				2		
Multifocal acute aspiration pneumonia						
slight				030013		
Score Expanded Totals				1		
Multifocal alveolar inflammatory cell infiltration						
very slight				030012		
Score Expanded Totals				1		
Focal osseous metaplasia						
slight				030012		
Score Expanded Totals				1		

Figures in brackets represent the number of animals from which this tissue was examined microscopically
The absence of a numeral indicates that the lesion specified was not identified

*** Listing Complete ***

Appendix 9 Light micrographs of lungs (15-week postexposure)

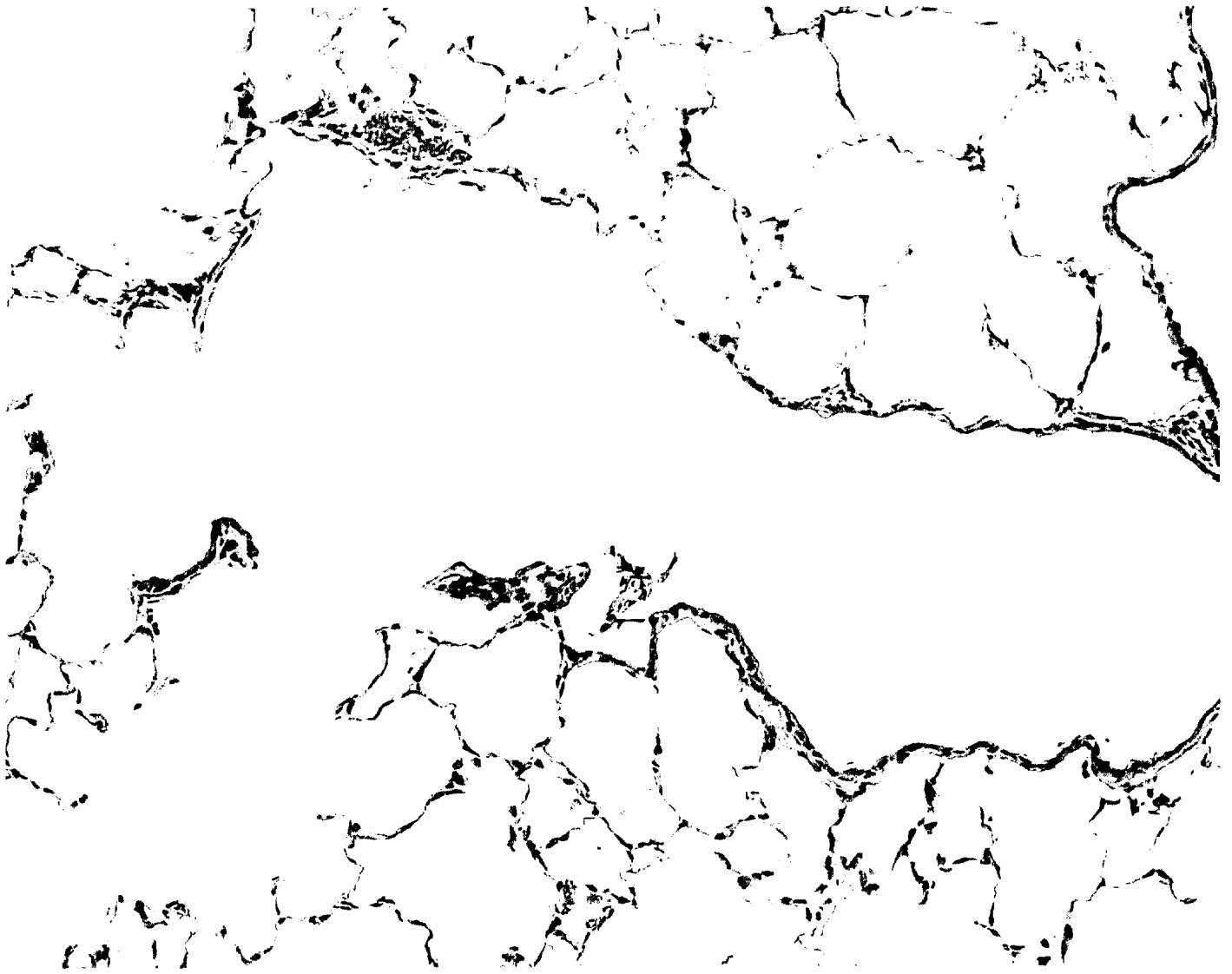


Fig. 1. Very slight (minimal) focal intra-bronchiolar accumulation of fibres associated with macrophages (animal no. 020011). H&E stain.

Appendix 9 (cont.) Light micrographs of lungs (15-week postexposure)

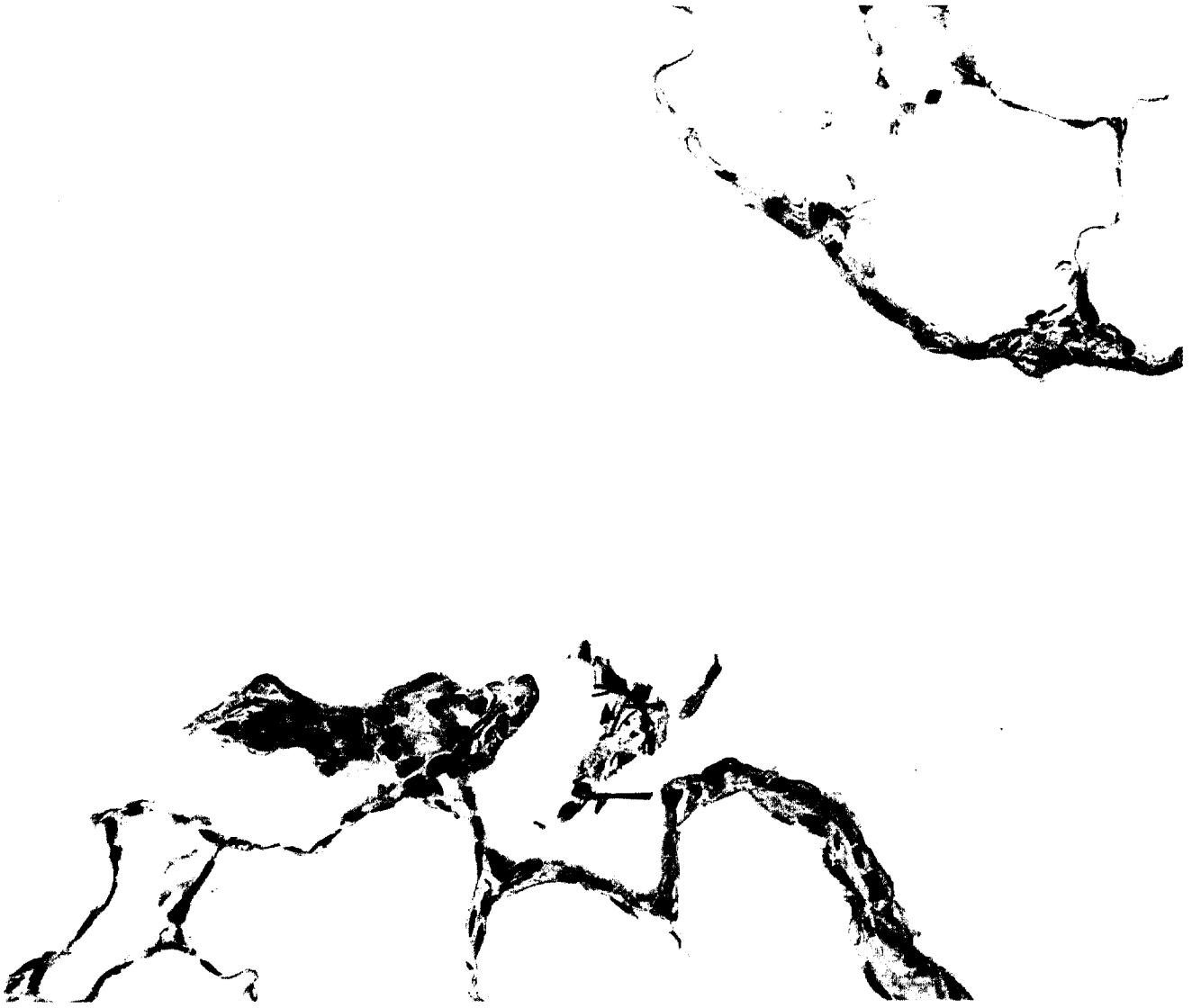


Fig. 2. Higher magnification of Fig. 1 (animal no. 020011). H&E stain.

Appendix 9 (cont.) Light micrographs of lungs (15-week postexposure)

Fig. 3. Very slight (minimal) focal accumulation of fibre-laden macrophages within alveolus/alveolar duct. One long fiber ist lying freely within alveolar lumen. Note very slight bronchiolo-alveolar hyperplasia (alveolar bronchiolization) of alveolar duct region (animal no. 020011). Masson trichrome stain.

Appendix 9 (cont.) Light micrographs of lungs (15-week postexposure)

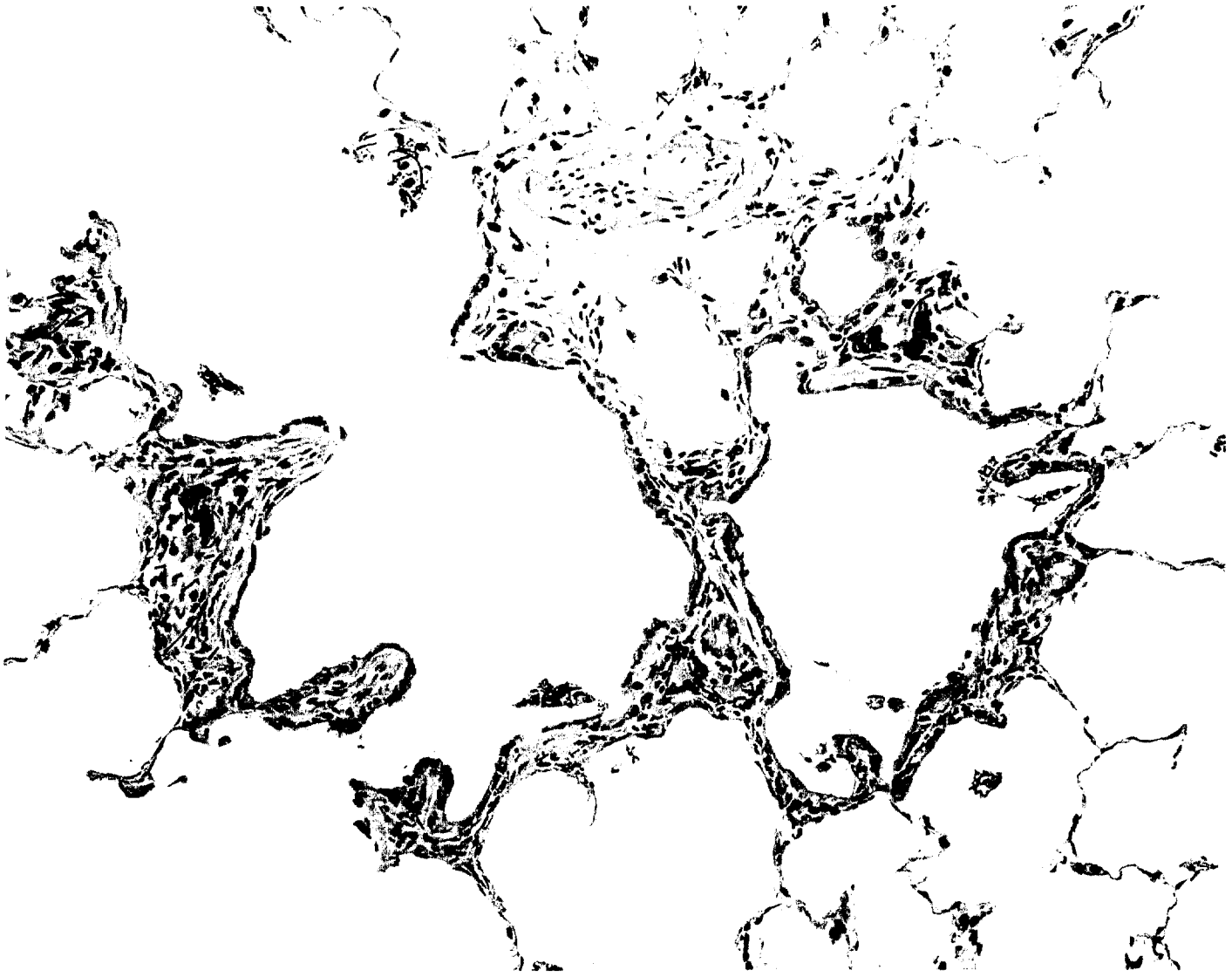


Fig. 4. Slight multifocal accumulation of fibre-laden macrophages within terminal bronchioles, alveoli and interstitium. Note slight bronchiolo-alveolar hyperplasia (alveolar bronchiolization) and slight formation of microgranulomas within interstitial areas (animal No. 030012). H&E stain

Appendix 9 (cont.) Light micrographs of lungs (15-week postexposure)

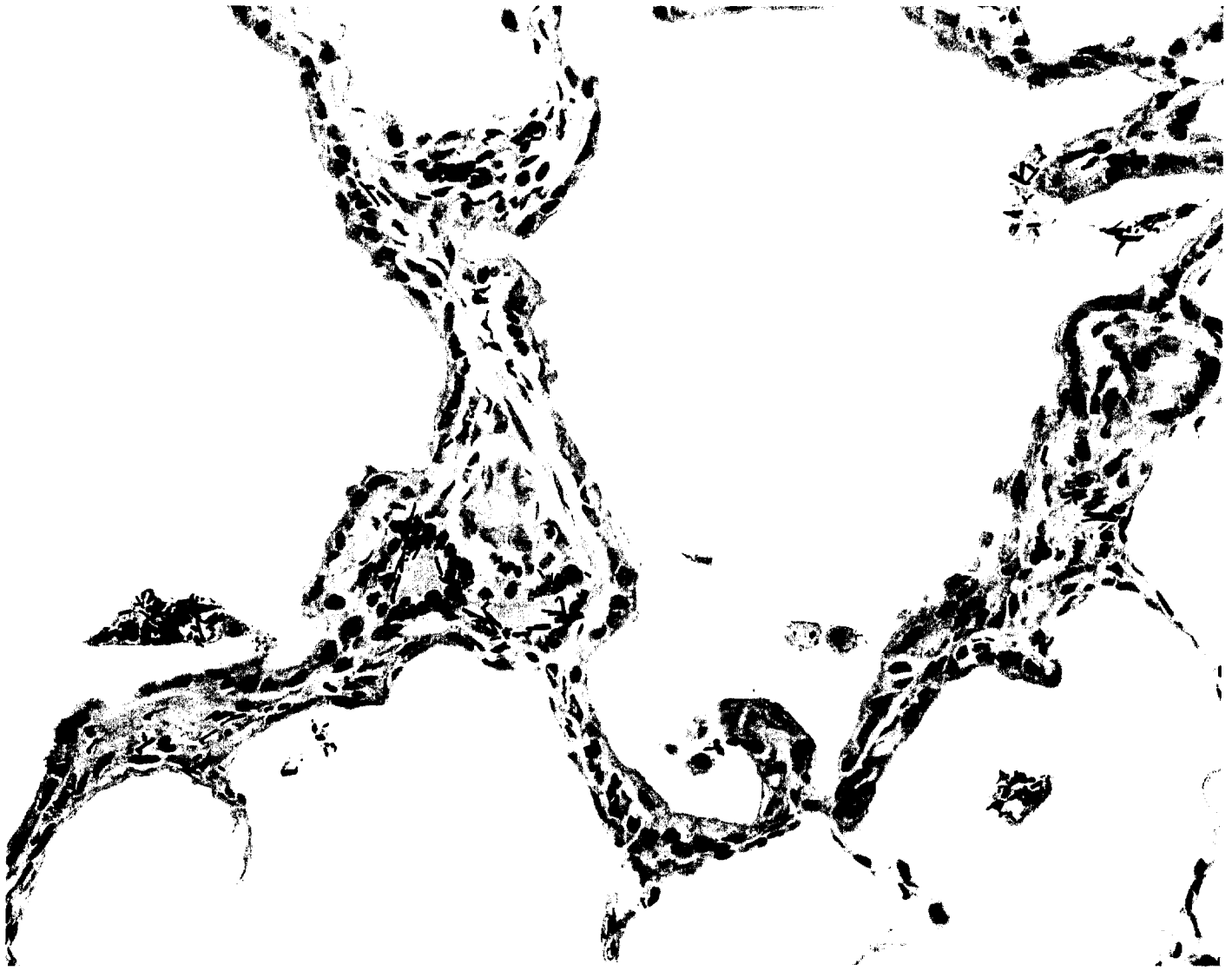


Fig. 5. Higher magnification of Fig. 4 (animal no. 030012). Some multinucleated giant cells associated with fibres are present within the interstitium. There is also evidence of very slight interstitial fibrosis in this area. H&E stain.

Appendix 9 (cont.) Light micrographs of lungs (15-week postexposure)

Fig. 6: The Masson trichrome stain shows slight focal interstitial fibrosis (top left) associated with microgranulomas (animal no. 030012).

Appendix 9 (cont.) Light micrographs of lungs (15-week postexposure)

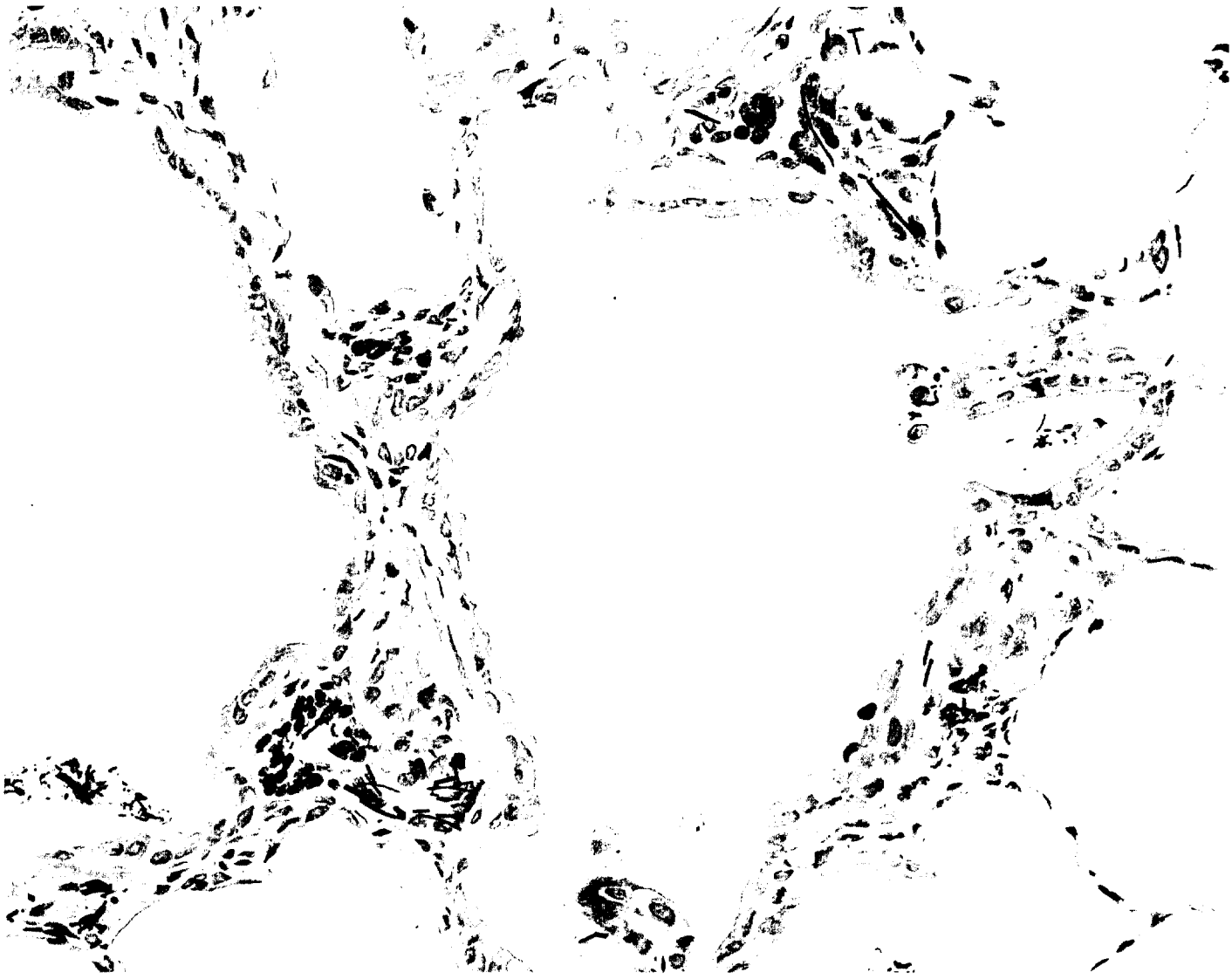


Fig. 7: Similar area as shown in Fig. 5 (animal no. 030012). Masson trichrome stain.

Appendix 9 (cont.) Light micrographs of lungs (15-week postexposure)

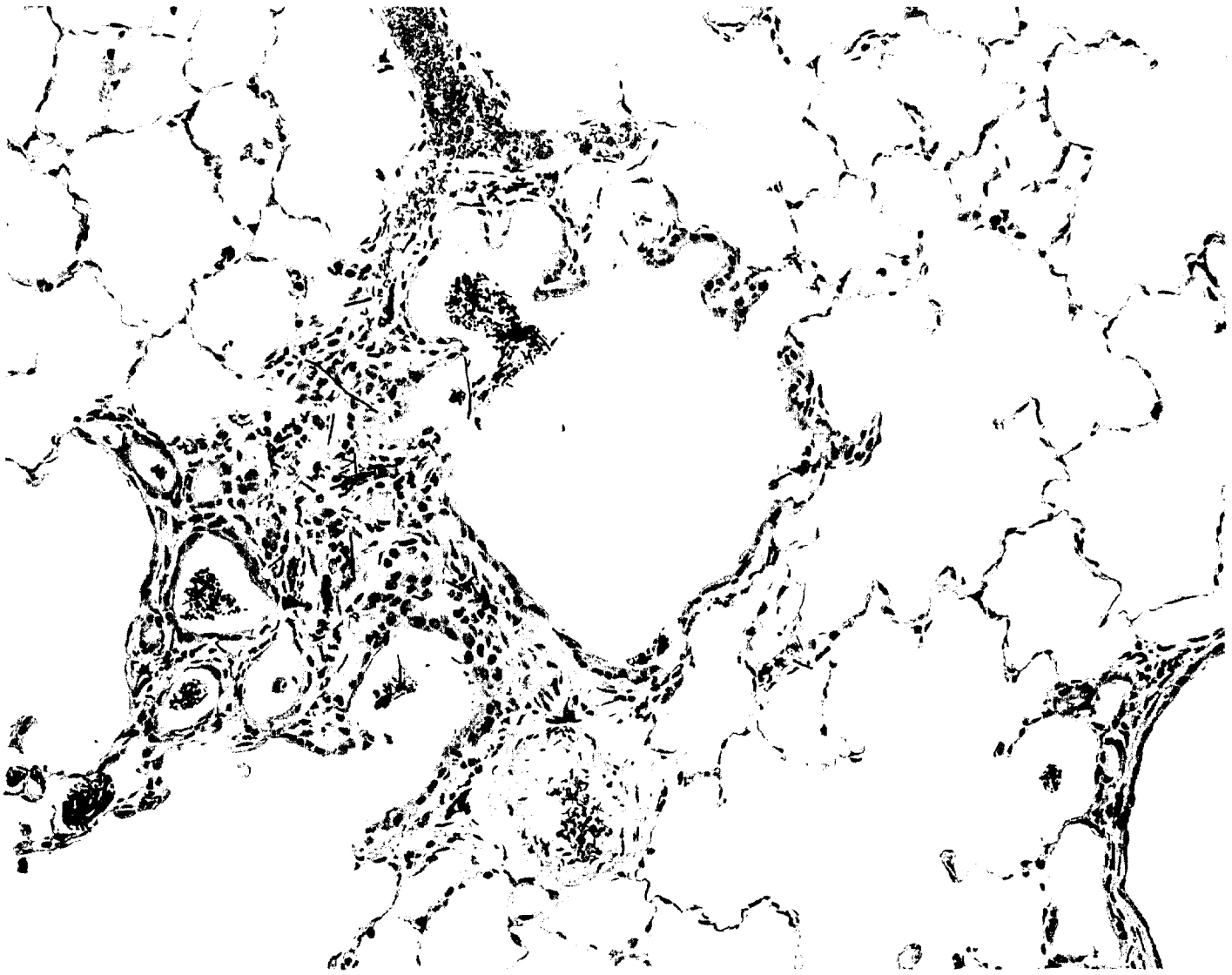


Fig. 8: Moderate amounts of fibres and fibre-laden macrophages within airways and interstitial areas, moderate formation of microgranulomas, slight multifocal bronchiolo-alveolar hyperplasia and slight multifocal interstitial fibrosis (animal no. 040012). H&E stain.

Appendix 9 (cont.) Light micrographs of lungs (15-week postexposure)

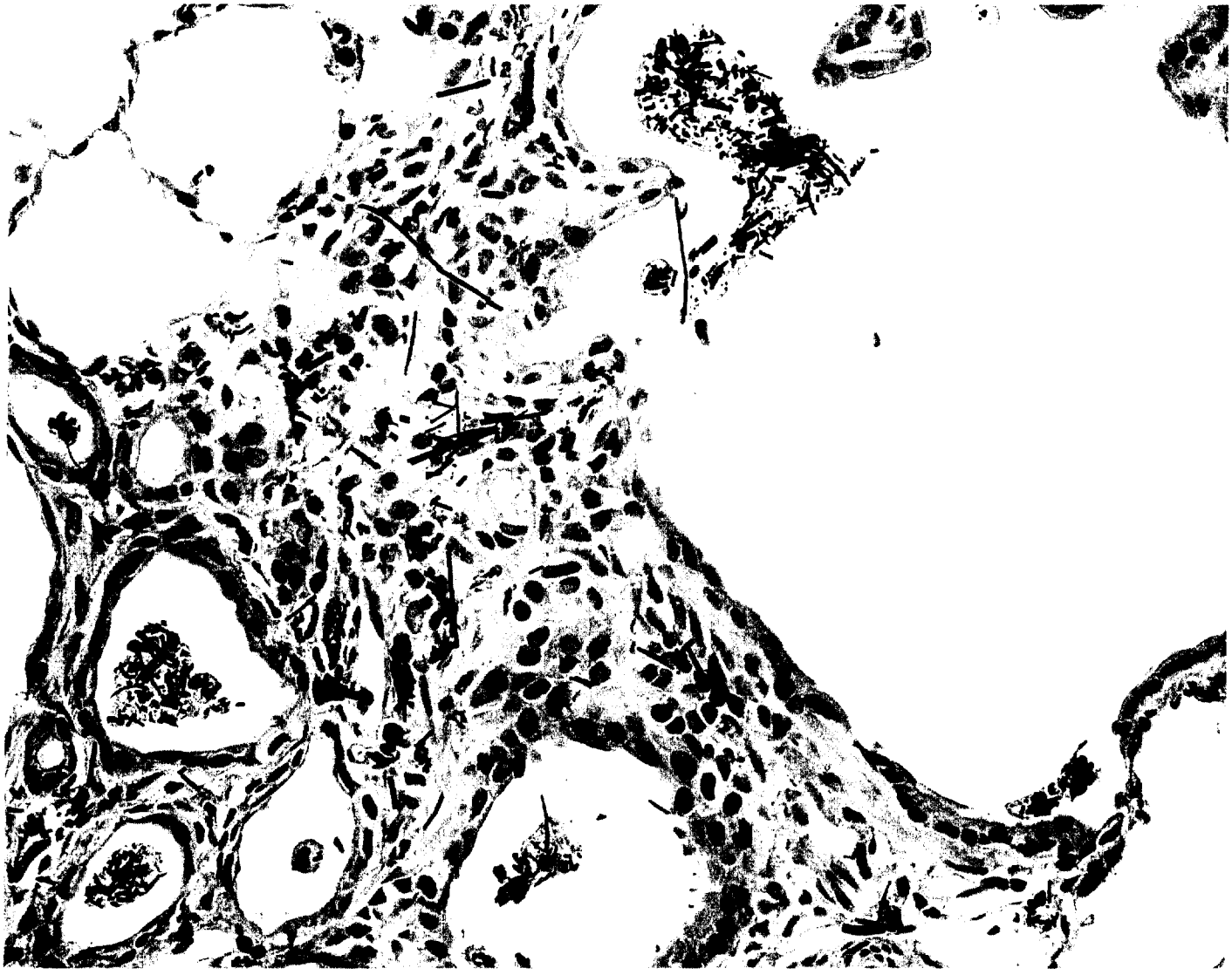


Fig. 9: Higher magnification of Fig. 8 (animal 040012). H&E stain.

Appendix 9 (cont.) Light micrographs of lungs (15-week postexposure)

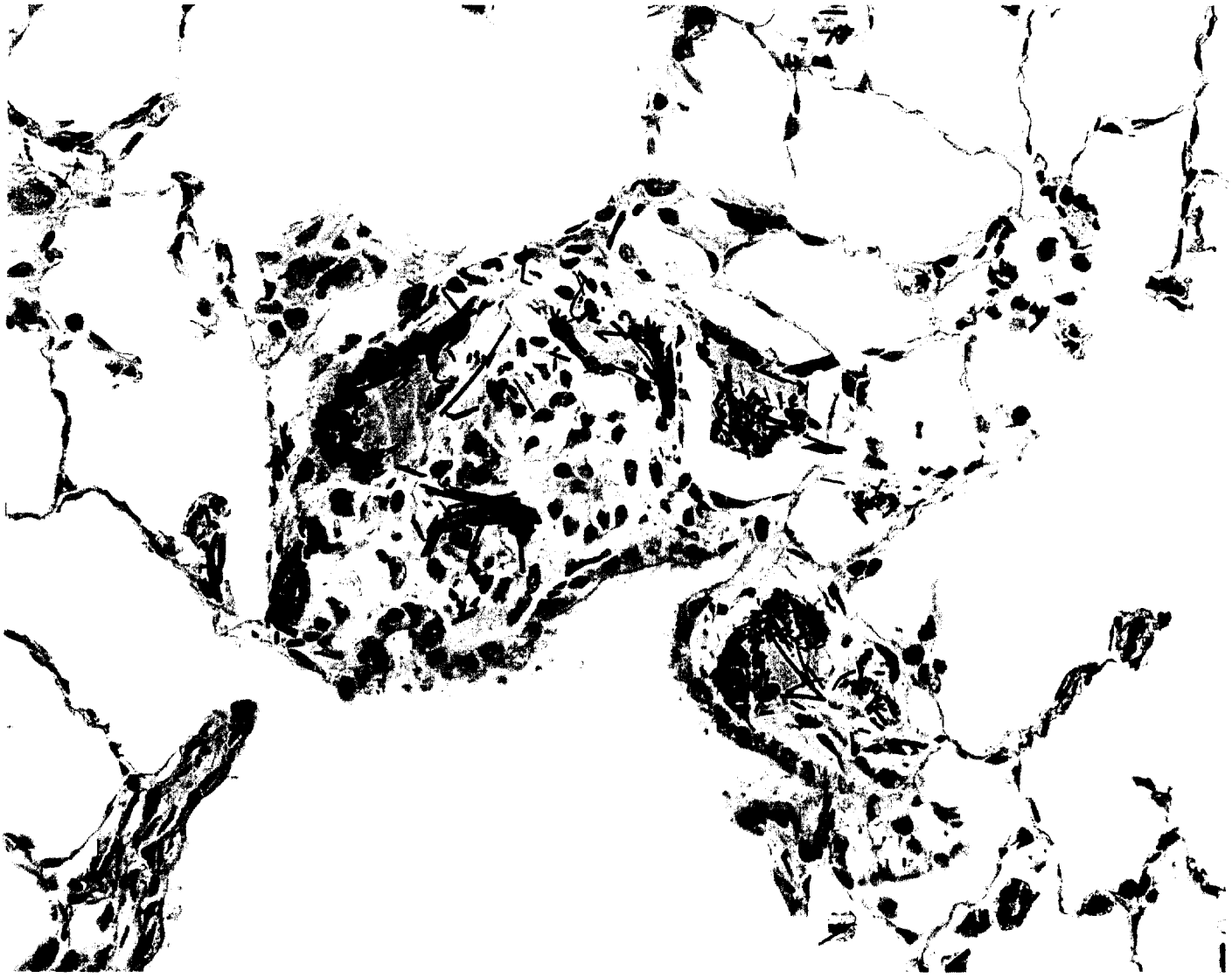


Fig 10. Numerous multinucleated giant cells associated with fibres of different sizes are present within the interstitium (animal no. 040012). H&E stain.

Appendix 9 (cont.) Light micrographs of lungs (15-week postexposure)

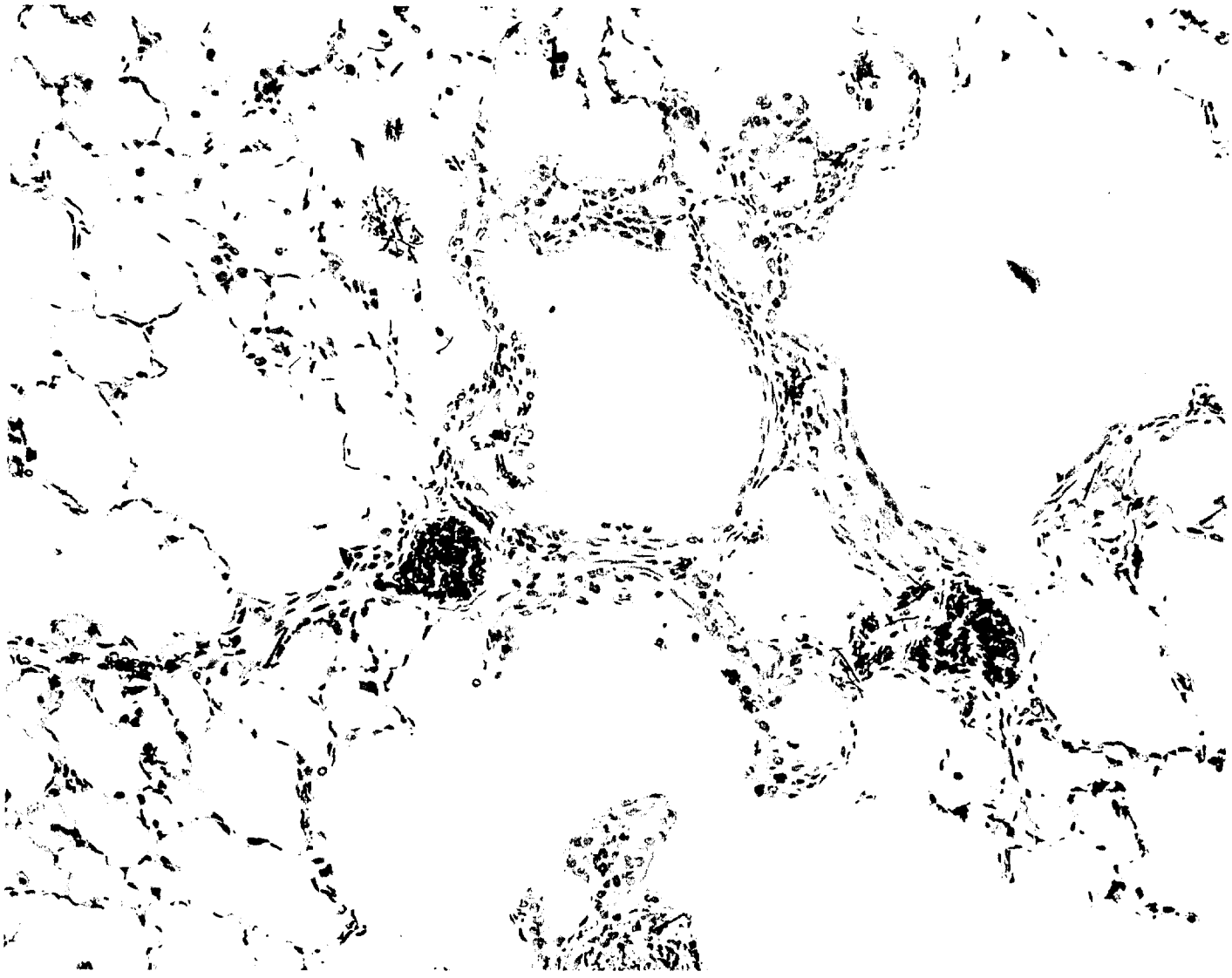


Fig. 11. Area of slight multifocal bronchiolo-alveolar hyperplasia and slight interstitial fibrosis in a rat lung from the high dose group (animal no. 040012). Masson trichrome stain.

Appendix 9 (cont.) Light micrographs of lungs (15-week postexposure)

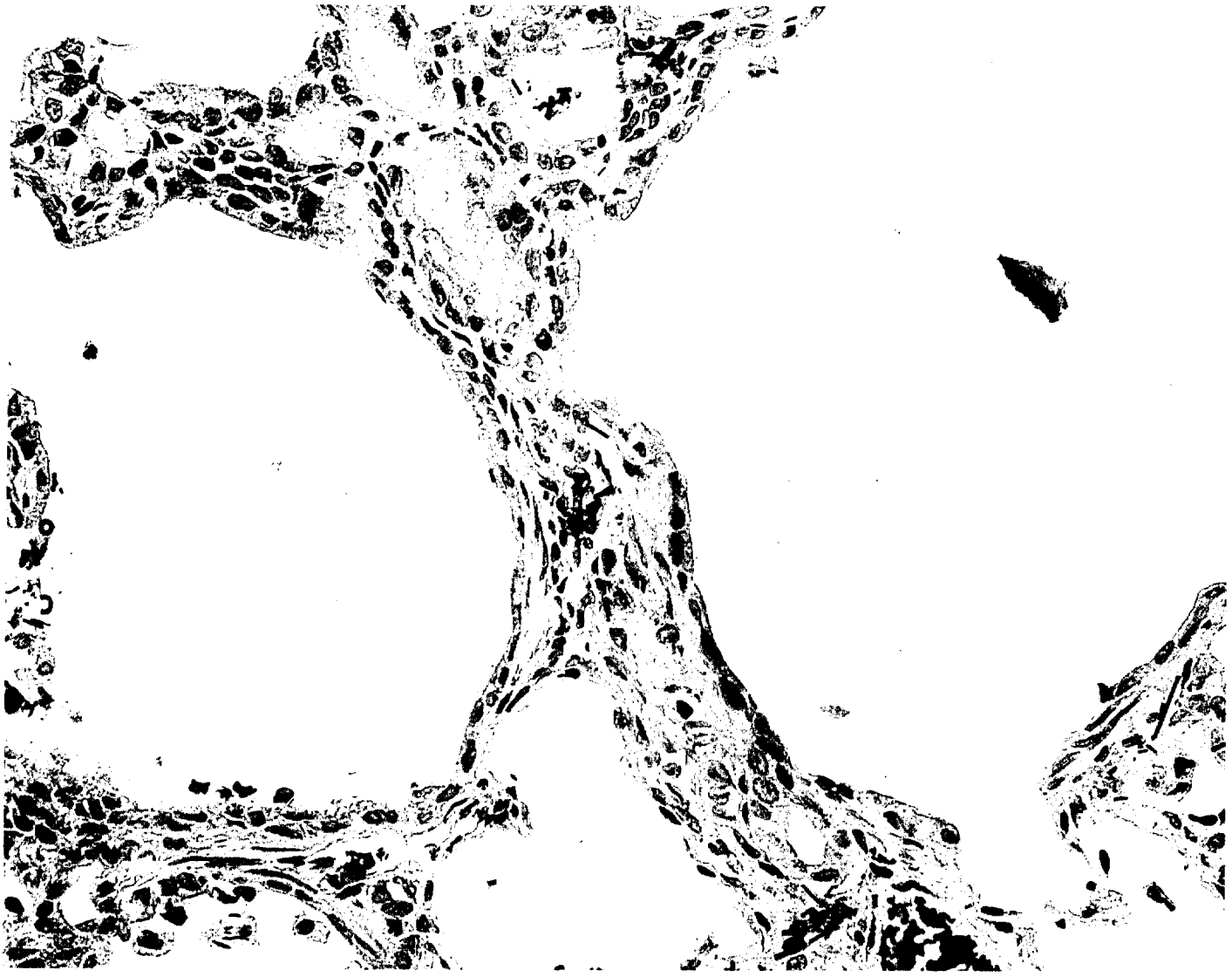
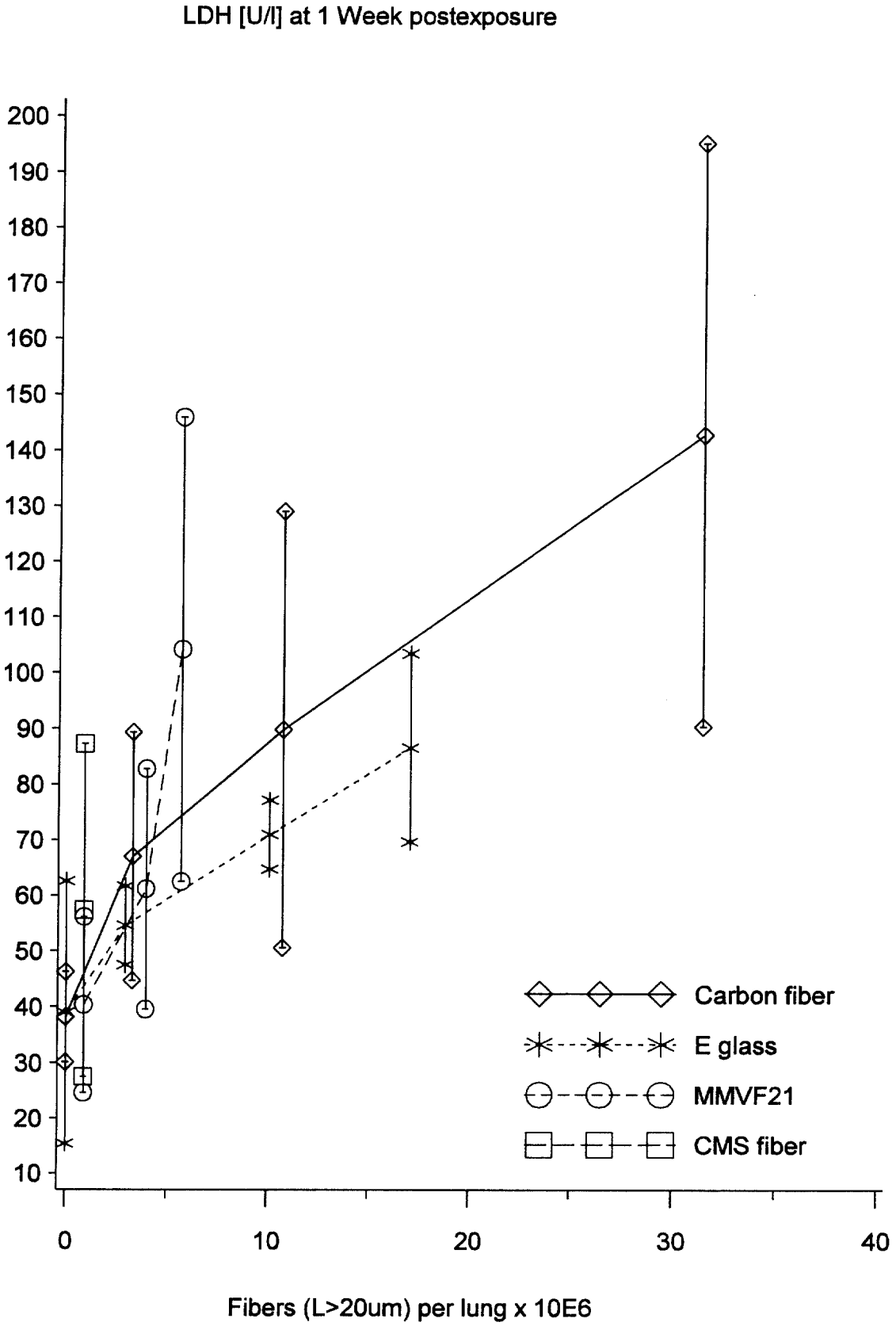
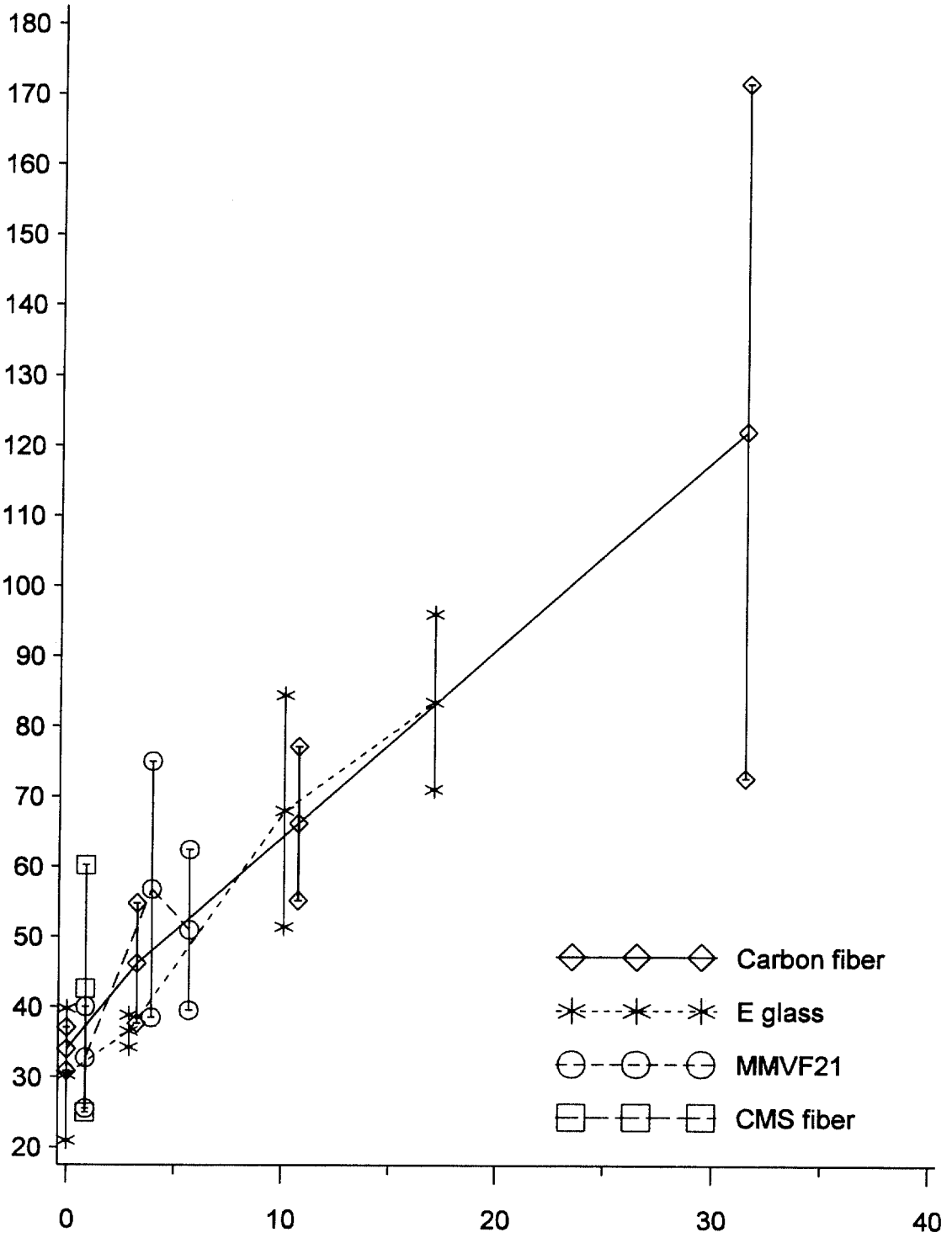


Fig. 12. Higher magnification of Fig. 11 (animal 040012). Masson trichrome stain.

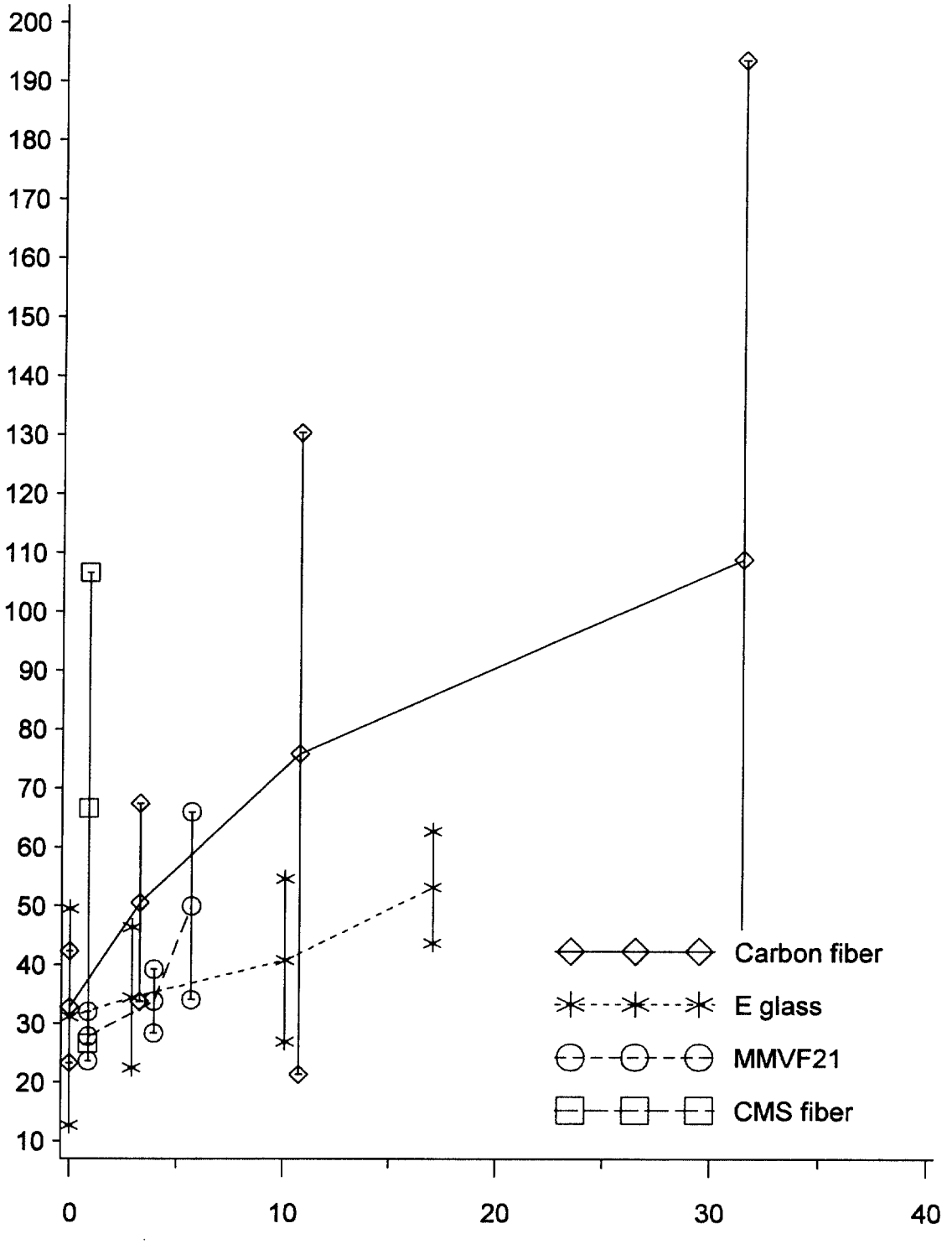
Appendix 10 Comparison of effects induced by carbon fibers with those of E glass, MMVF21 and CMS in a previous Fraunhofer ITEM study



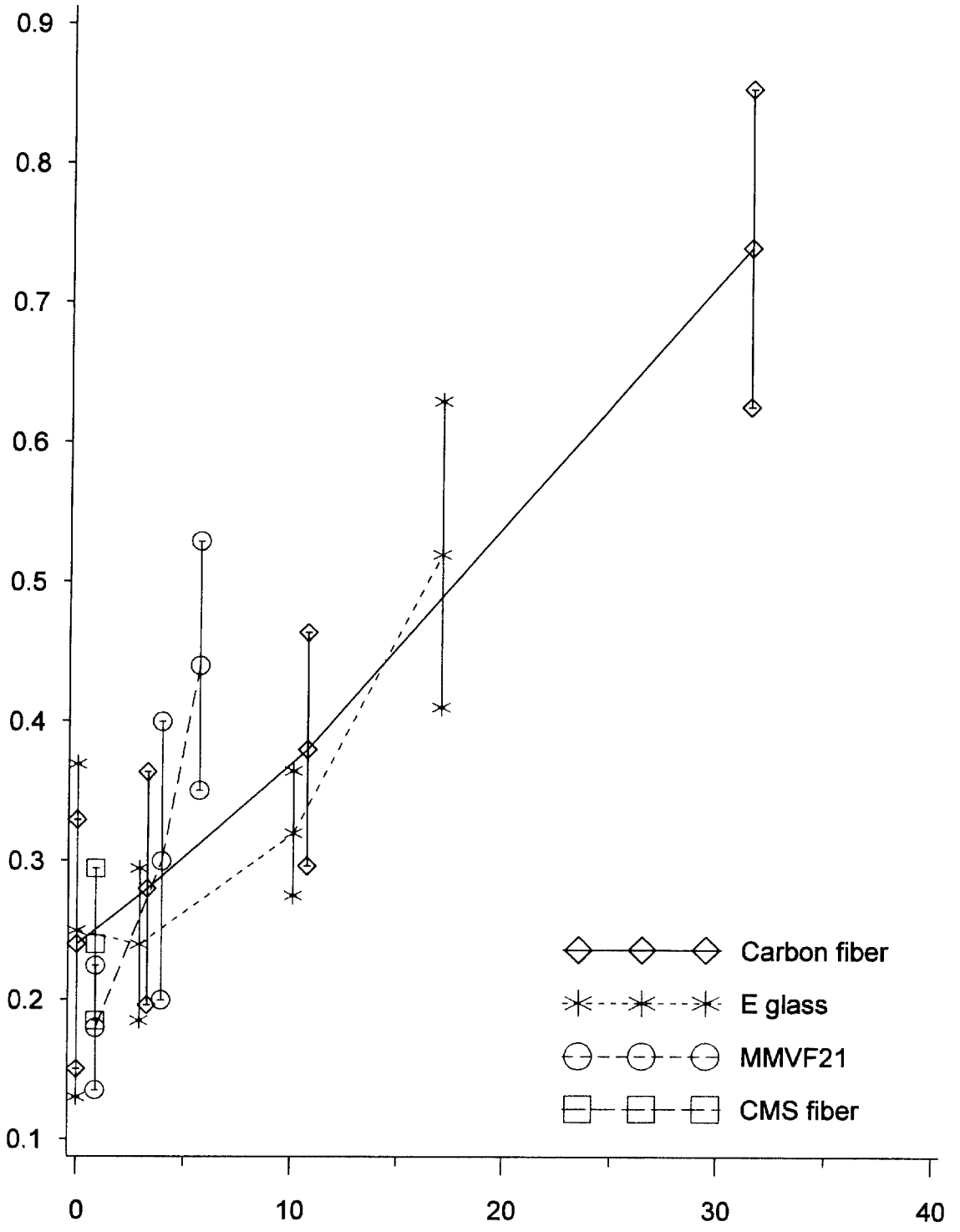
LDH [U/l] at 8 Weeks postexposure



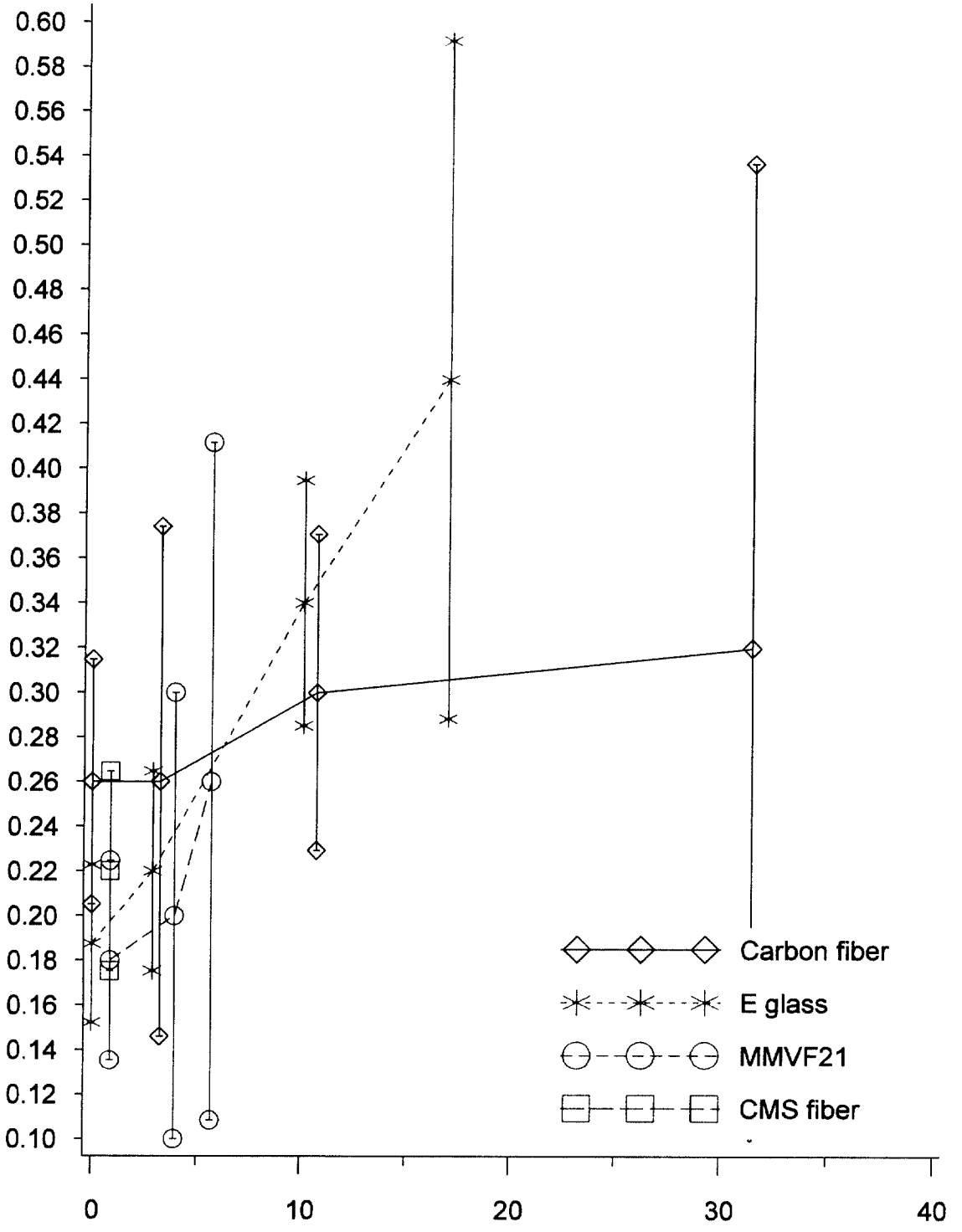
LDH [U/l] at 15 Weeks postexposure



ß-Glucuronidase [U/l] at 1 Week postexposure

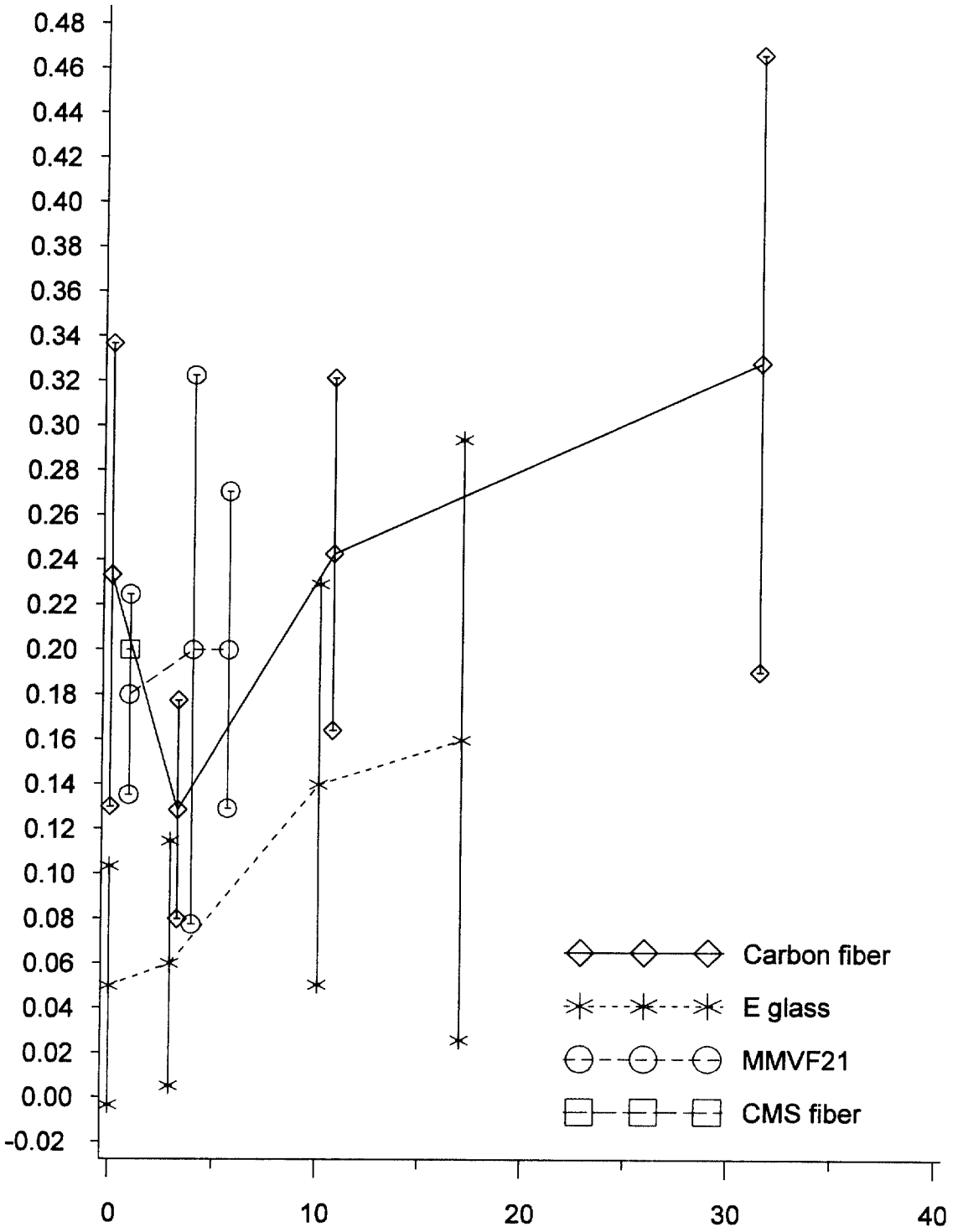


ß-Glucuronidase [U/l] at 8 Weeks postexposure



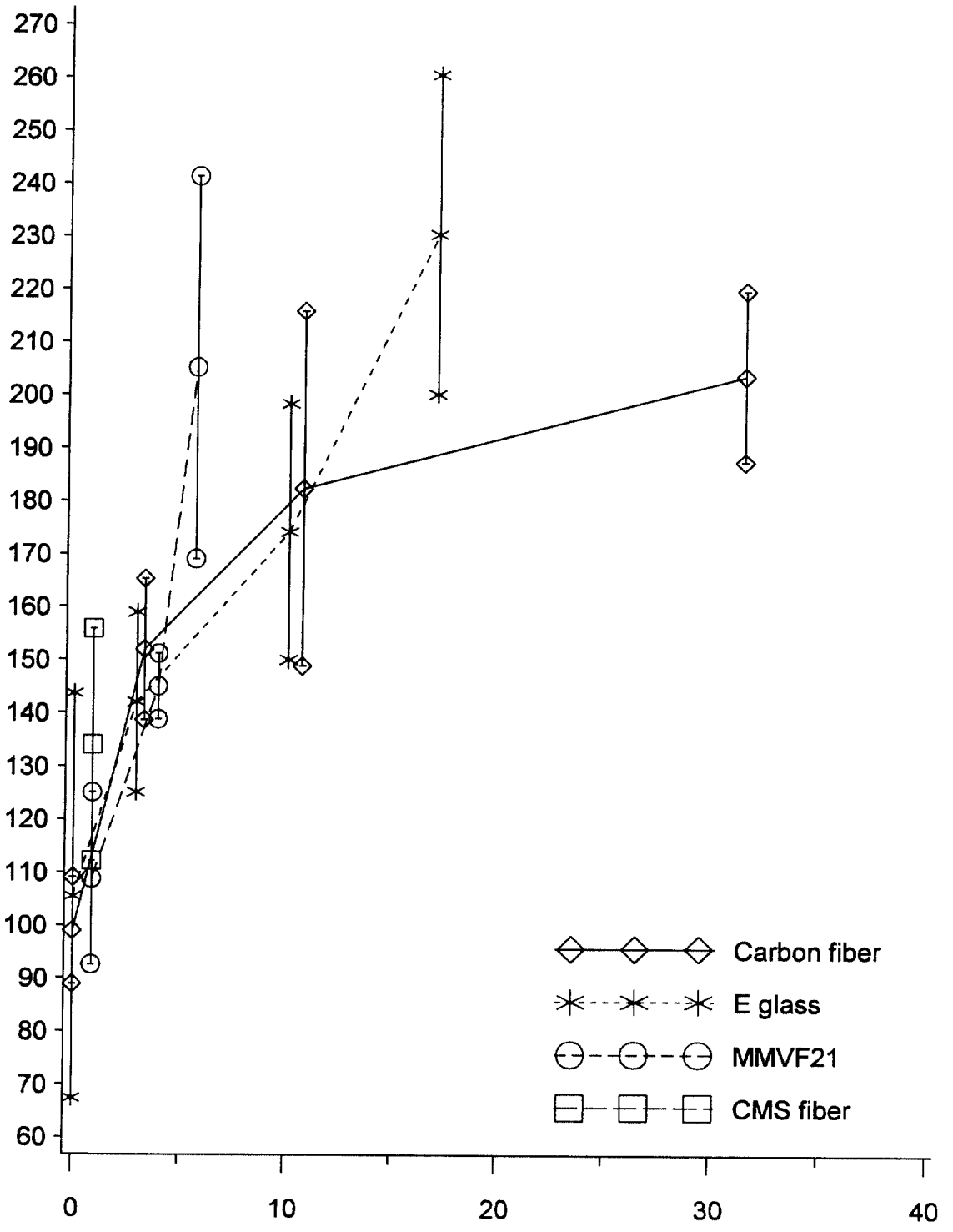
Fibers (L>20um) per lung x 10E6

ß-Glucuronidase [U/l] at 15 Weeks postexposure

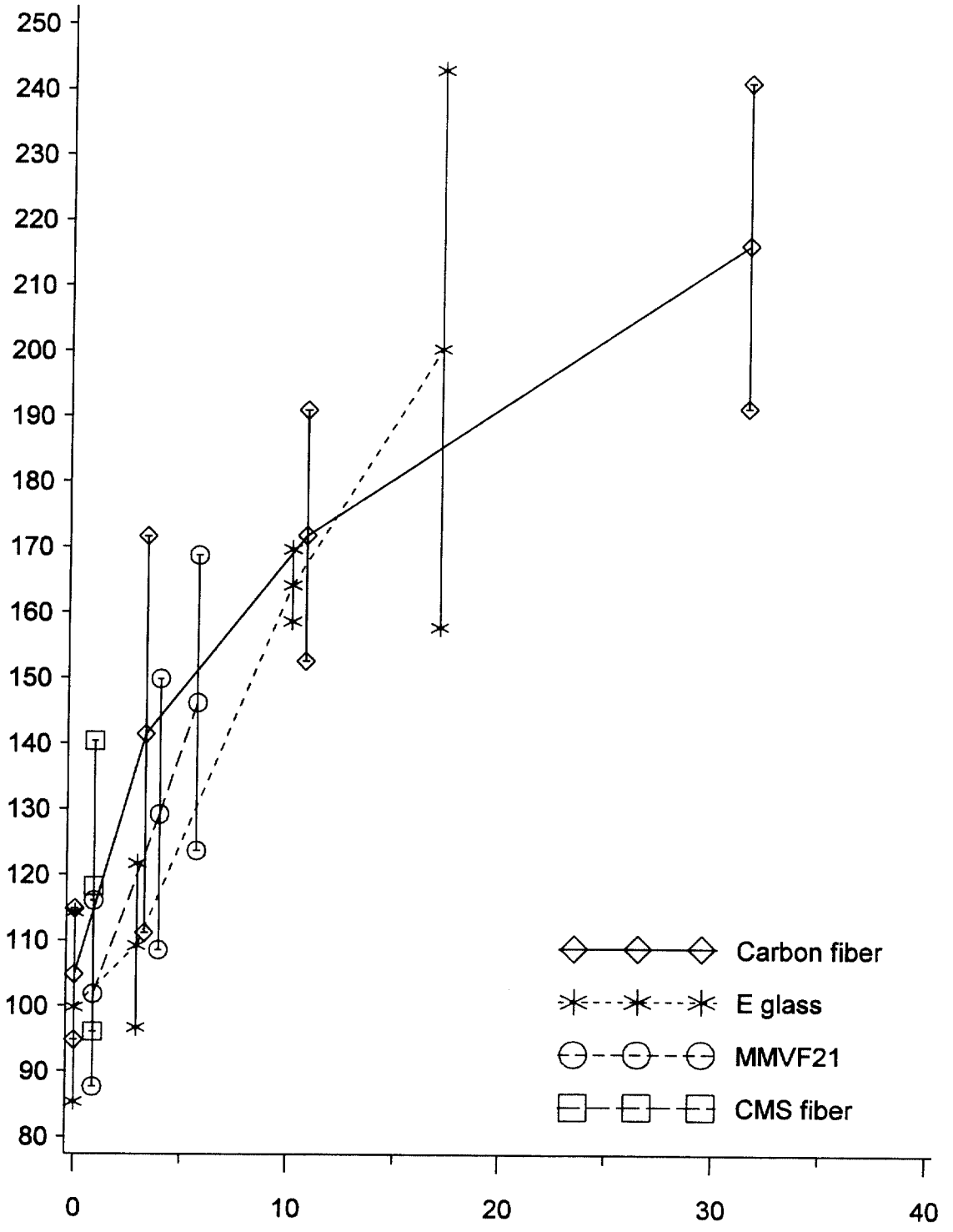


Fibers (L>20um) per lung x 10E6

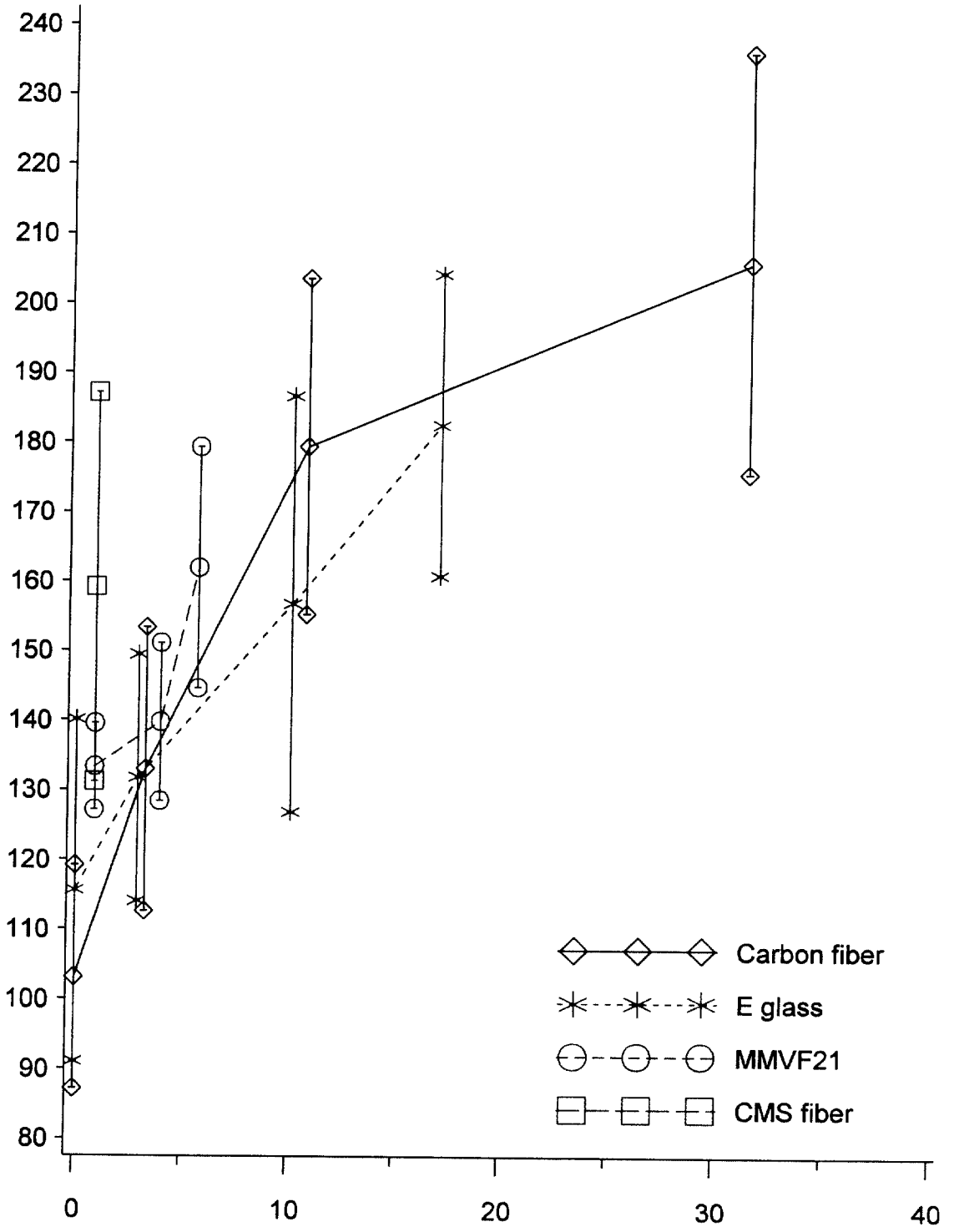
Total protein [mg/l] at 1 Week postexposure



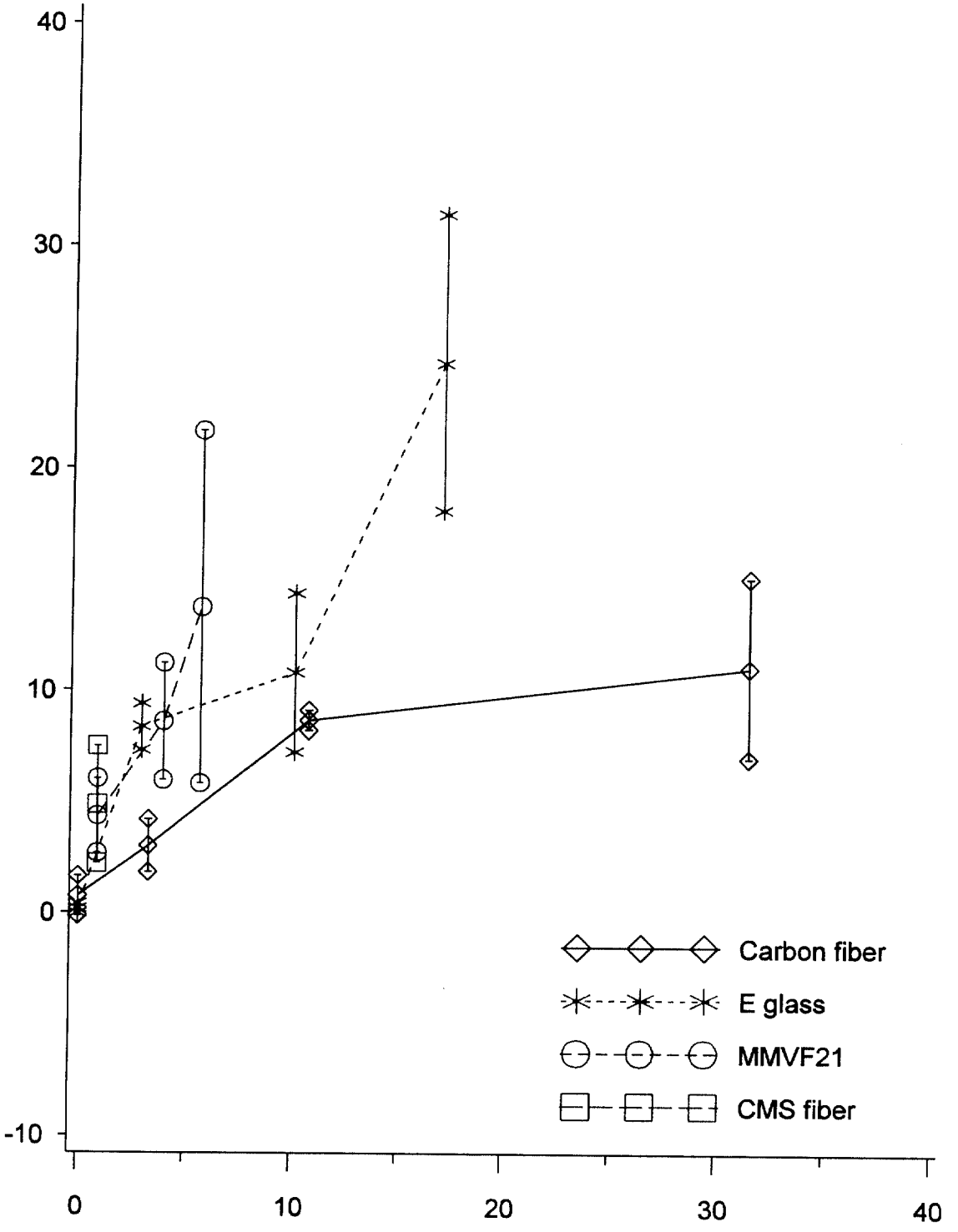
Total protein [mg/l] at 8 Weeks postexposure



Total protein [mg/l] at 15 Weeks postexposure

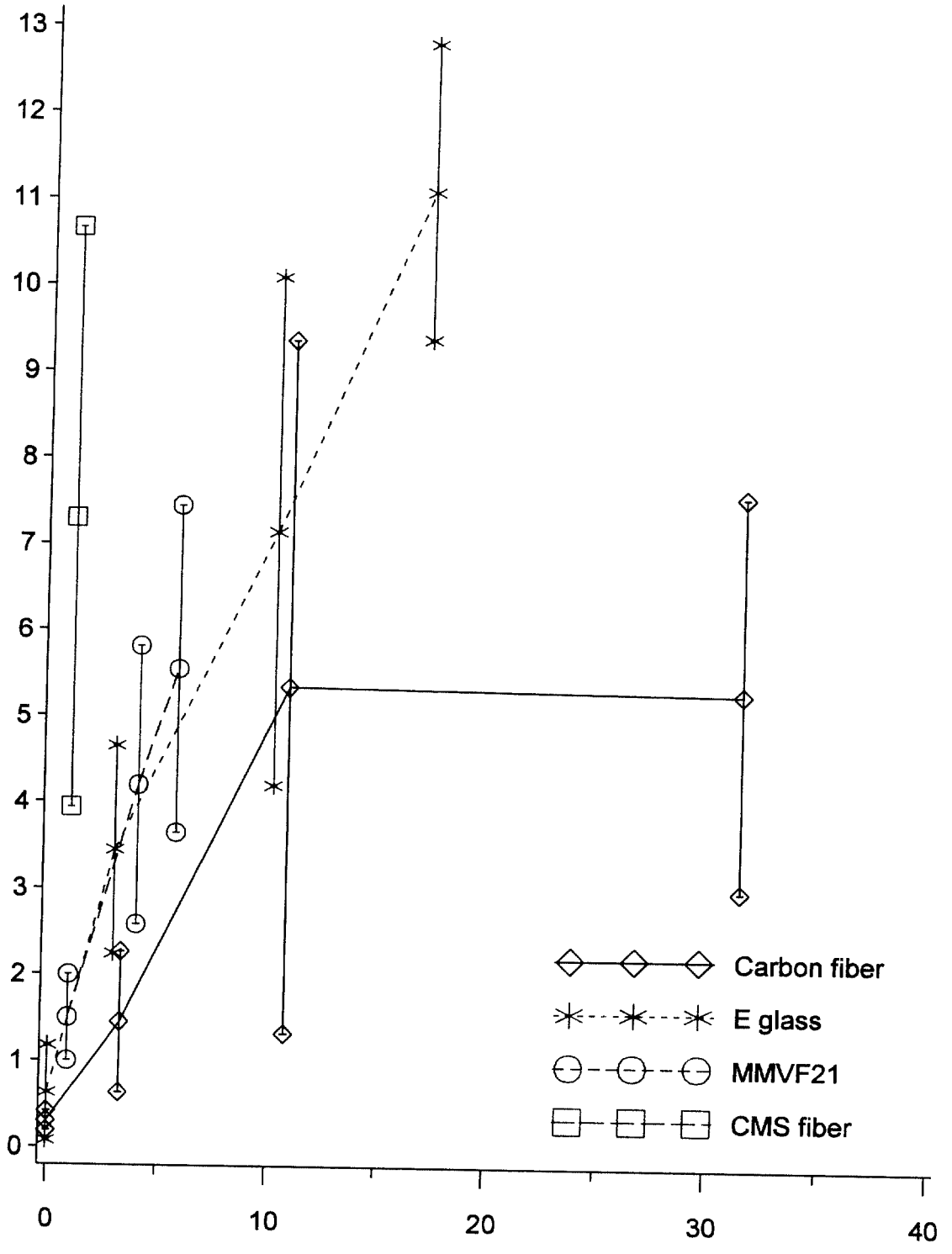


% PMNs at 1 Week postexposure



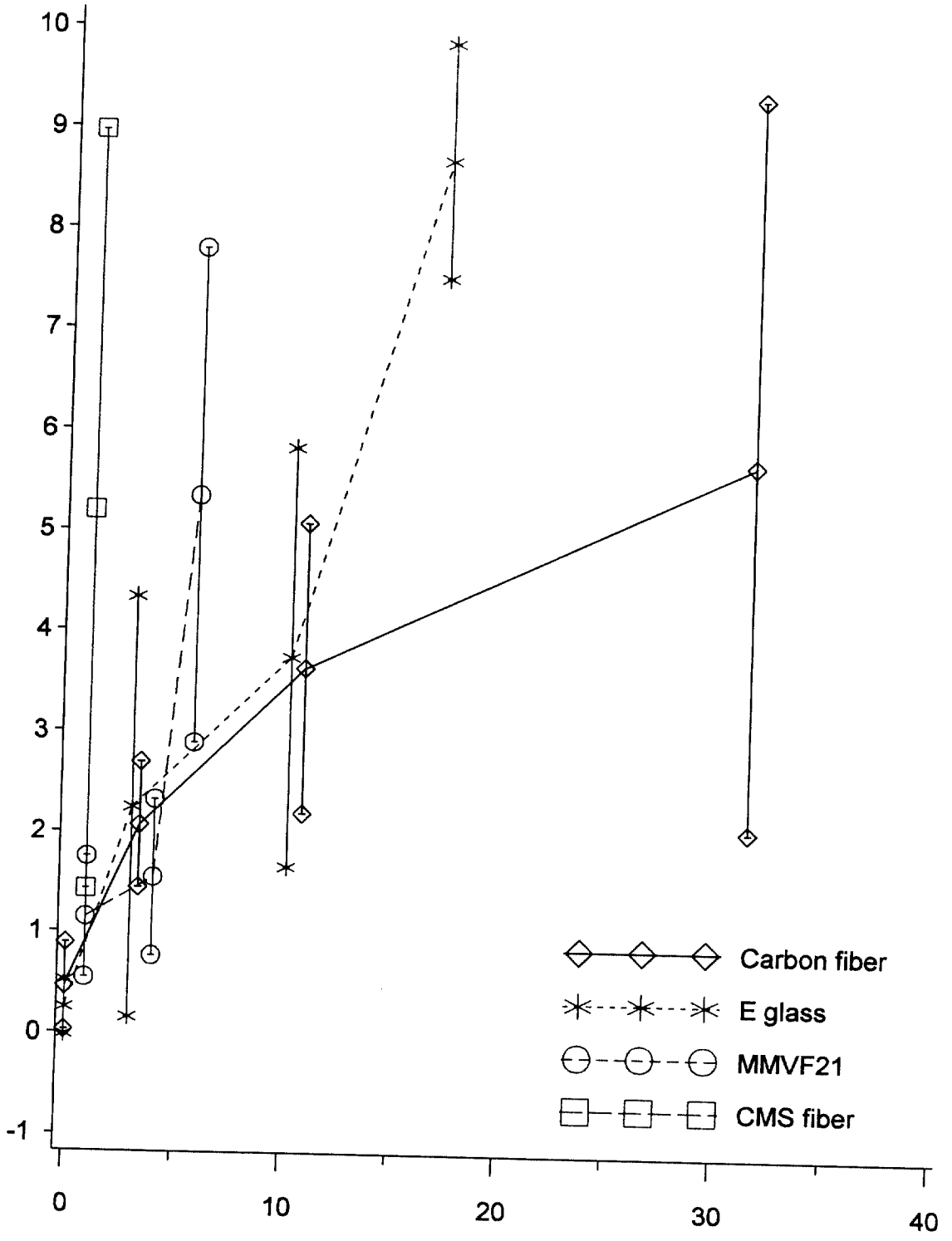
Fibers (L>20um) per lung x 10E6

% PMNs at 8 Weeks postexposure



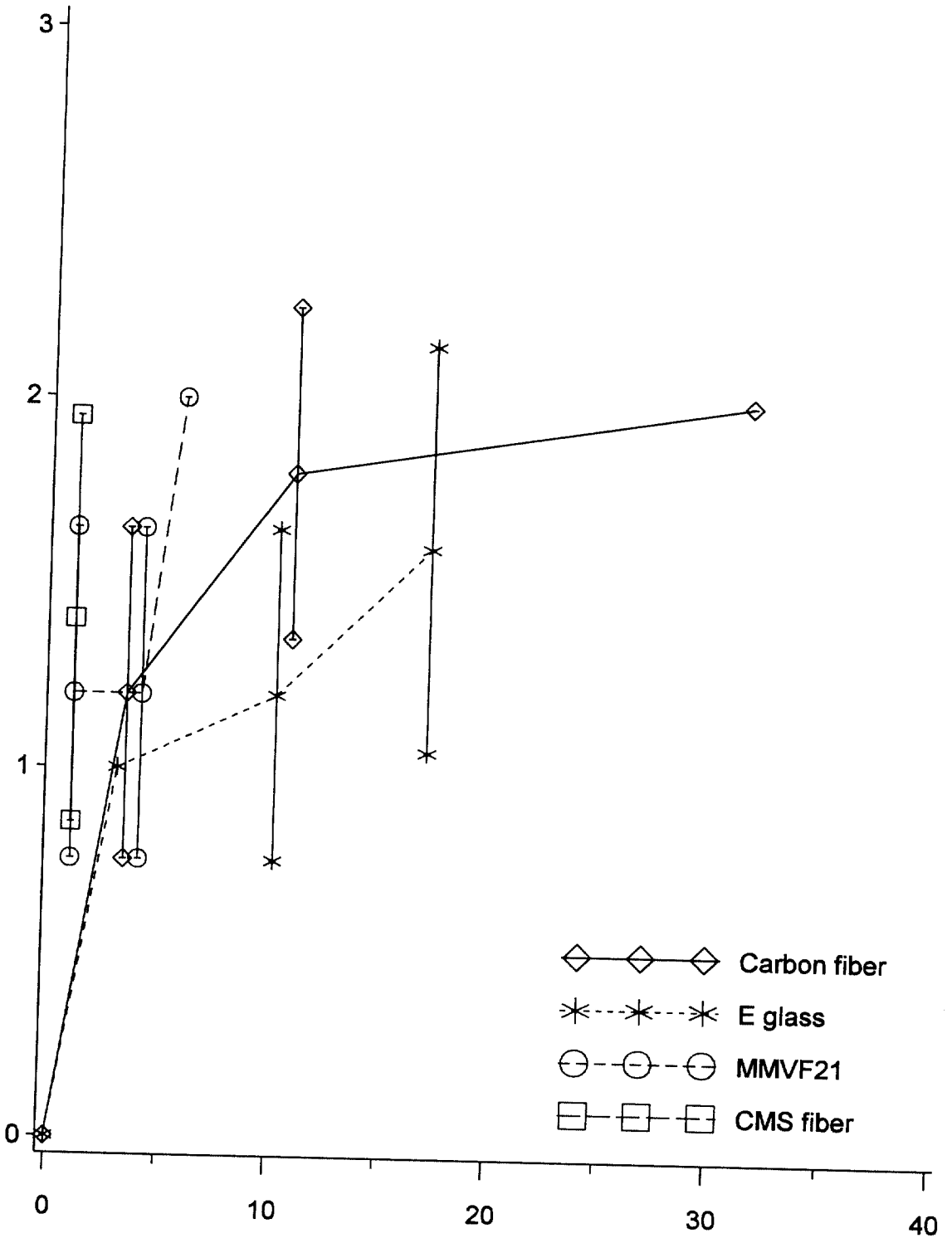
Fibers (L>20um) per lung x 10E6

% PMNs at 15 Weeks postexposure

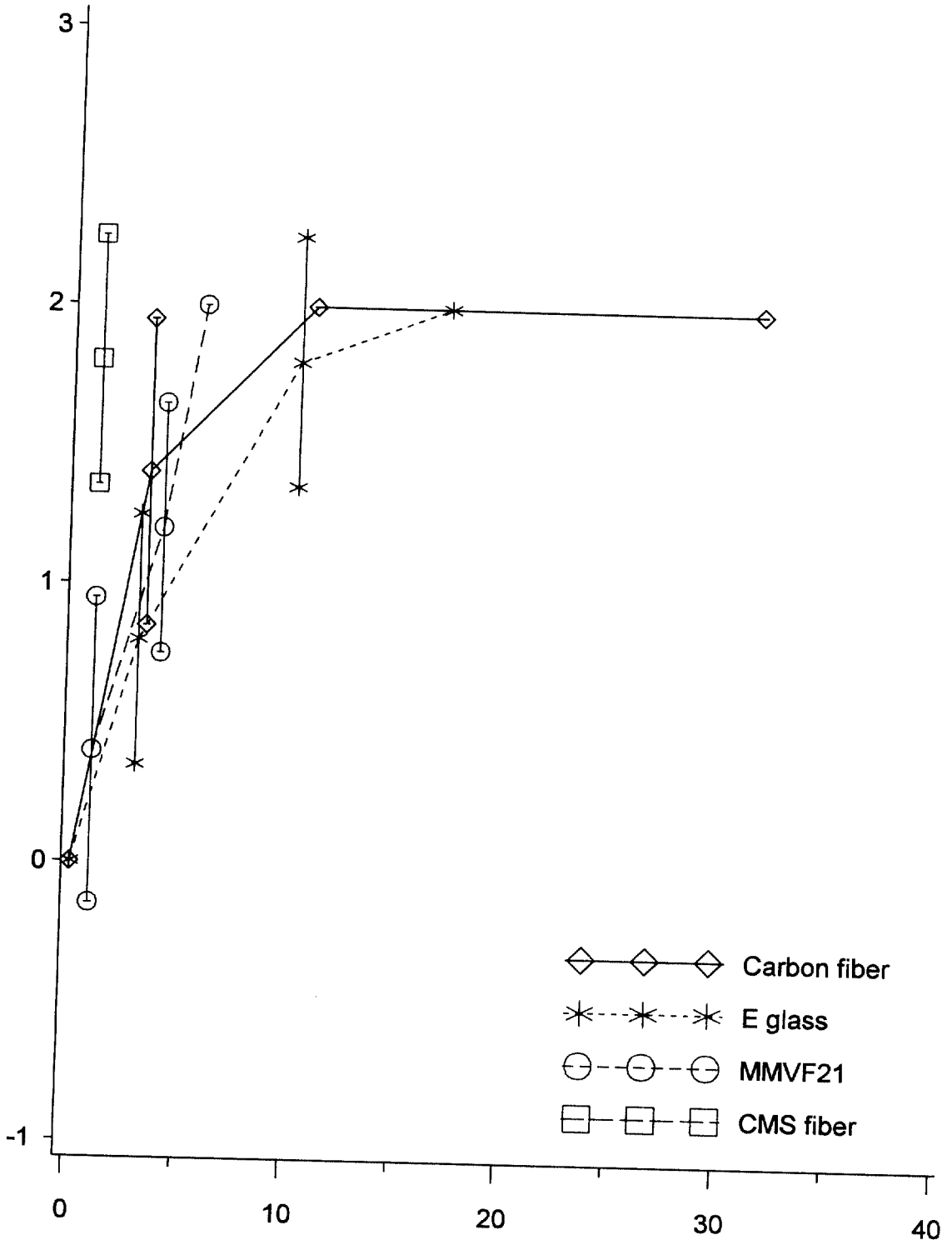


Fibers (L>20um) per lung x 10E6

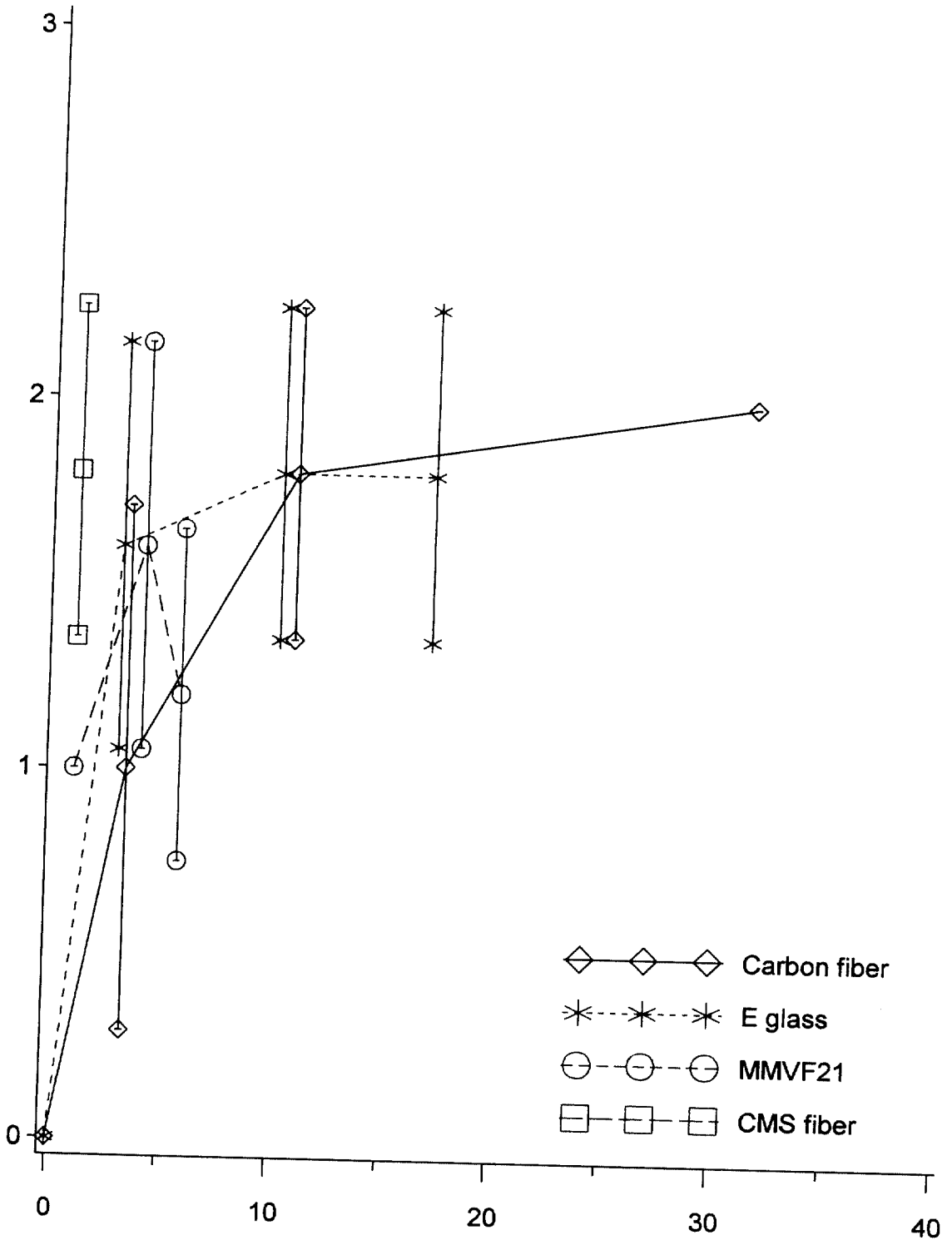
Bronchiolo-alveolar hperplasia at 1 Week postexposure



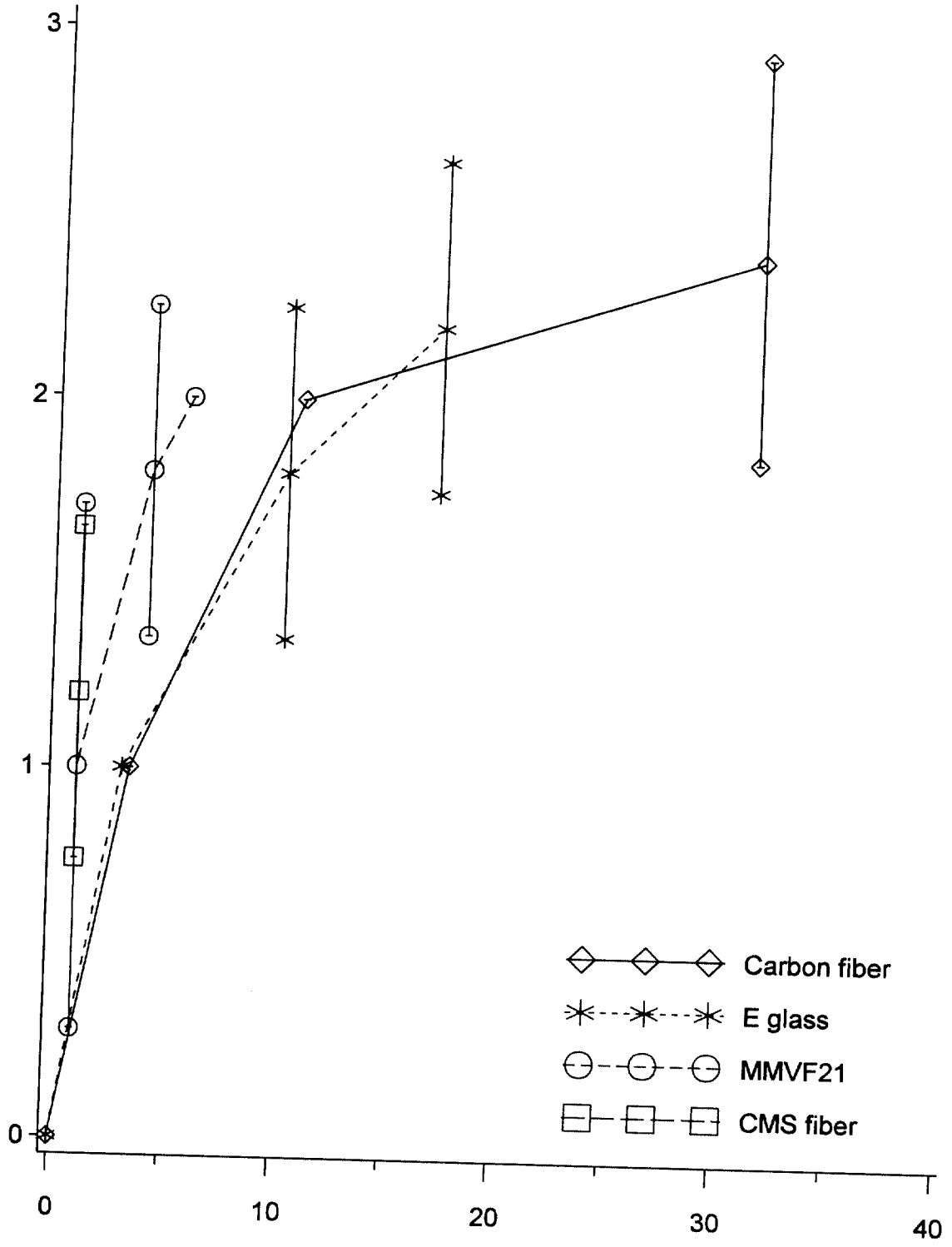
Bronchiolo-alveolar hperplasia at 8 Weeks postexposure



Bronchiolo-alveolar hperplasia at 15 Weeks postexposure

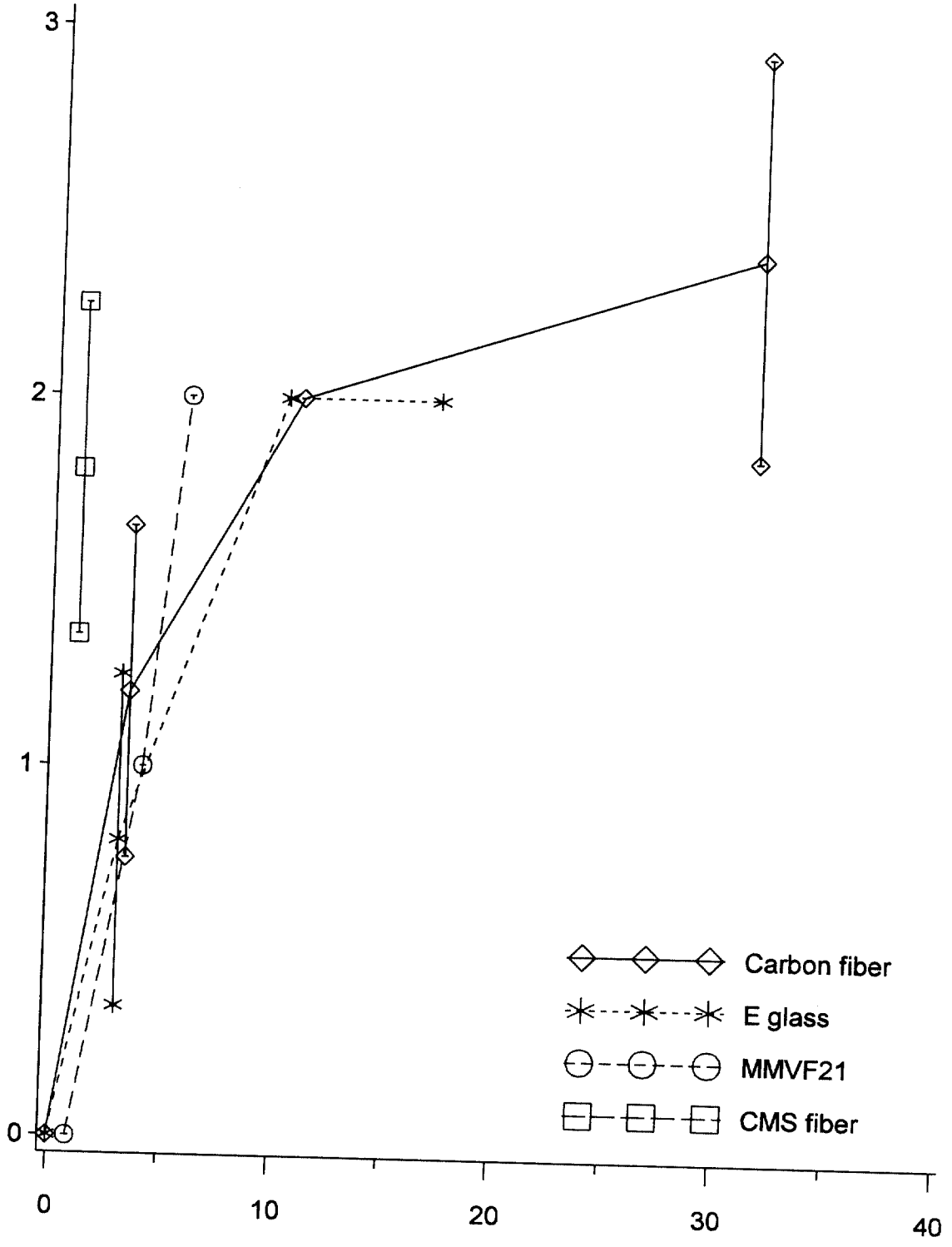


Mycrogranulomas at 1 Week postexposure

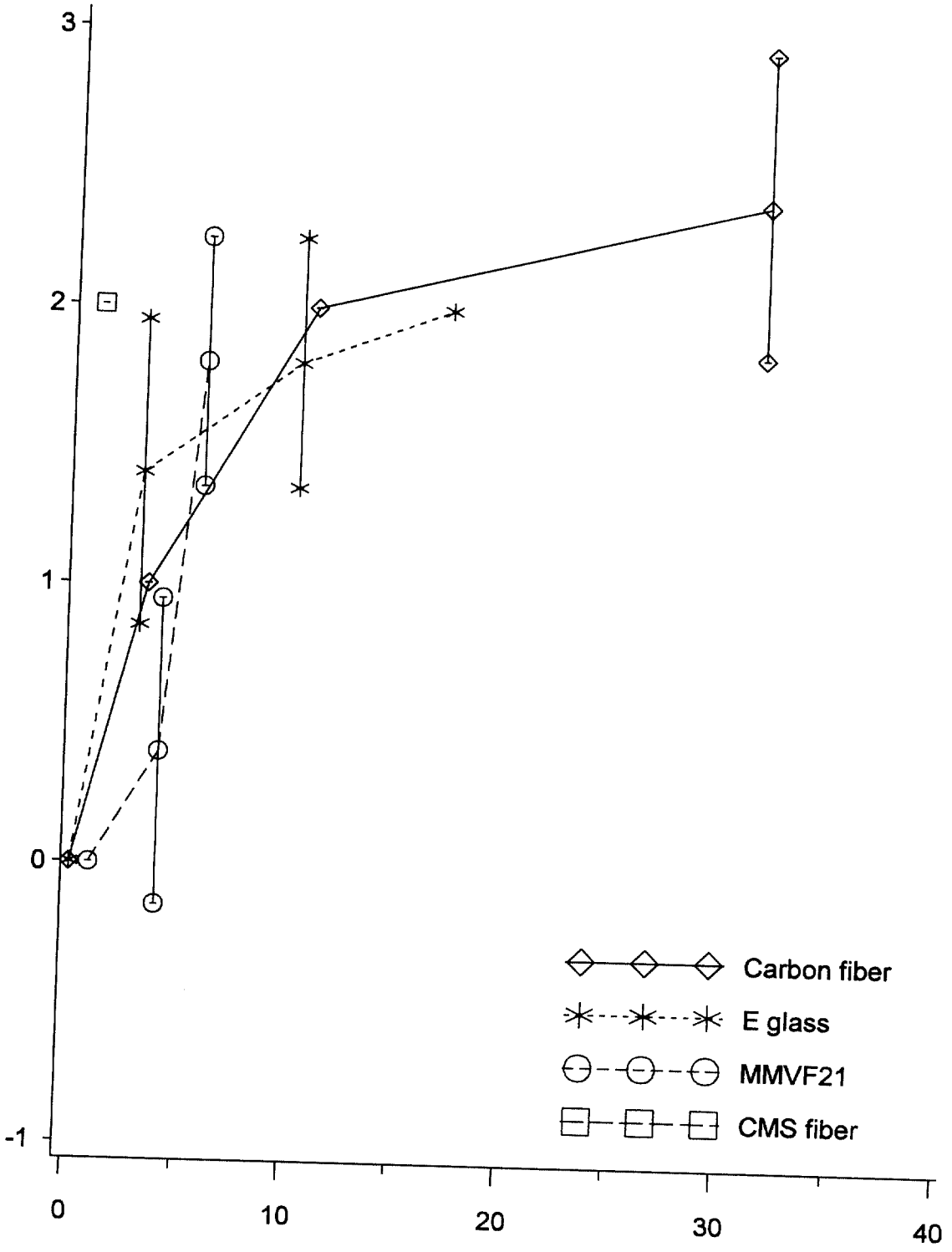


Fibers (L>20um) per lung x 10E6

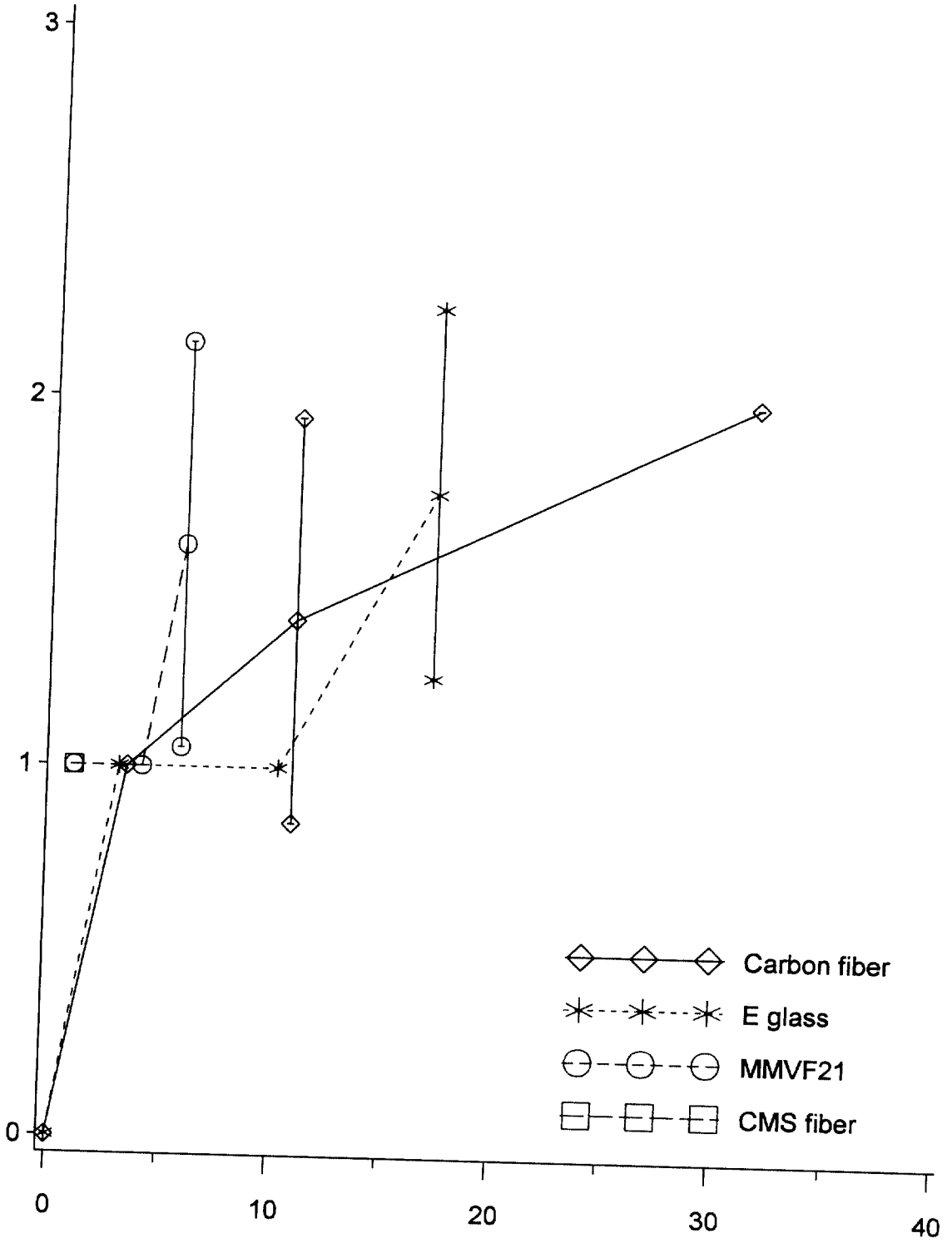
Mycrogranulomas at 8 Weeks postexposure



Mycrogranulomas at 15 Weeks postexposure

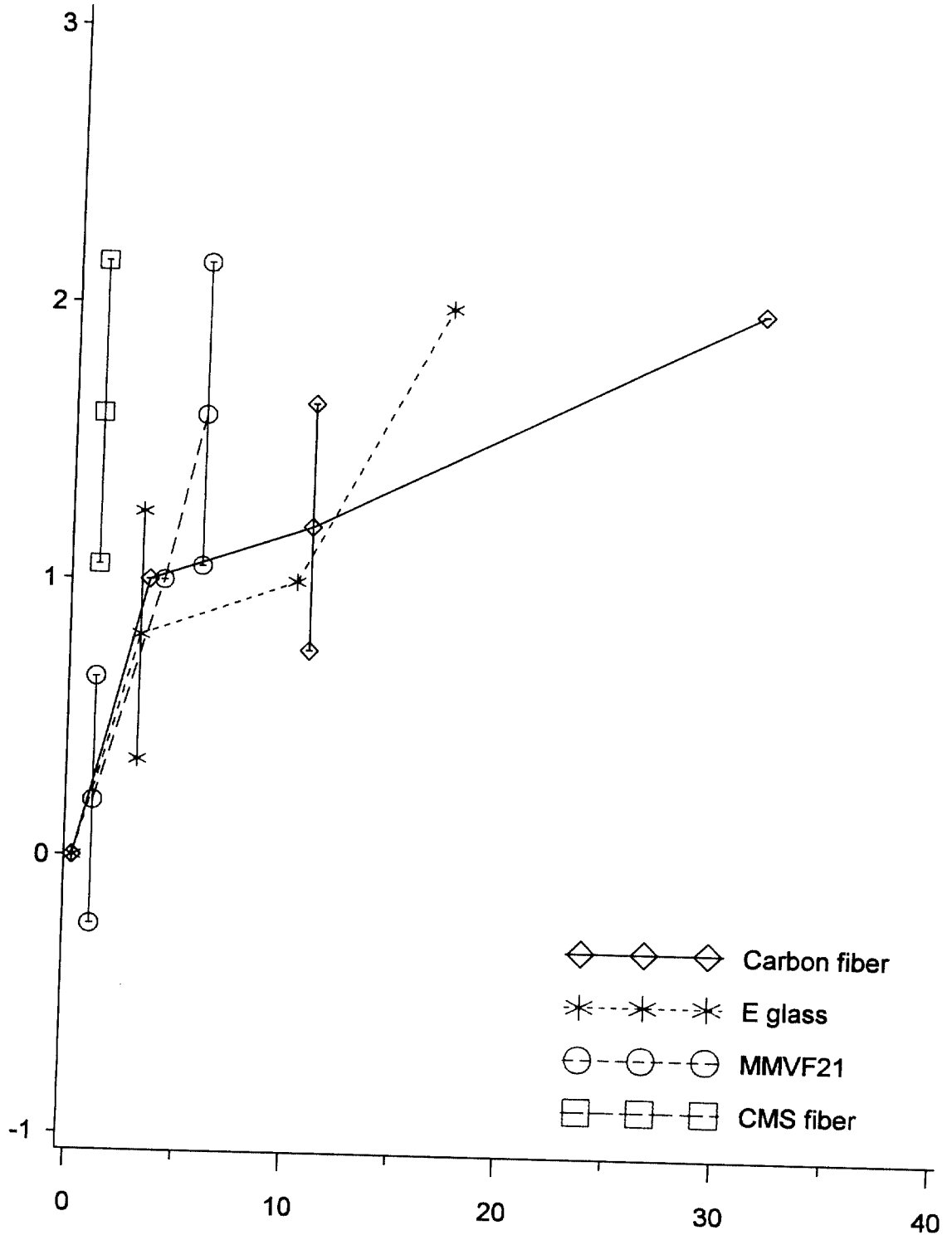


EPS grade at 1 Week postexposure



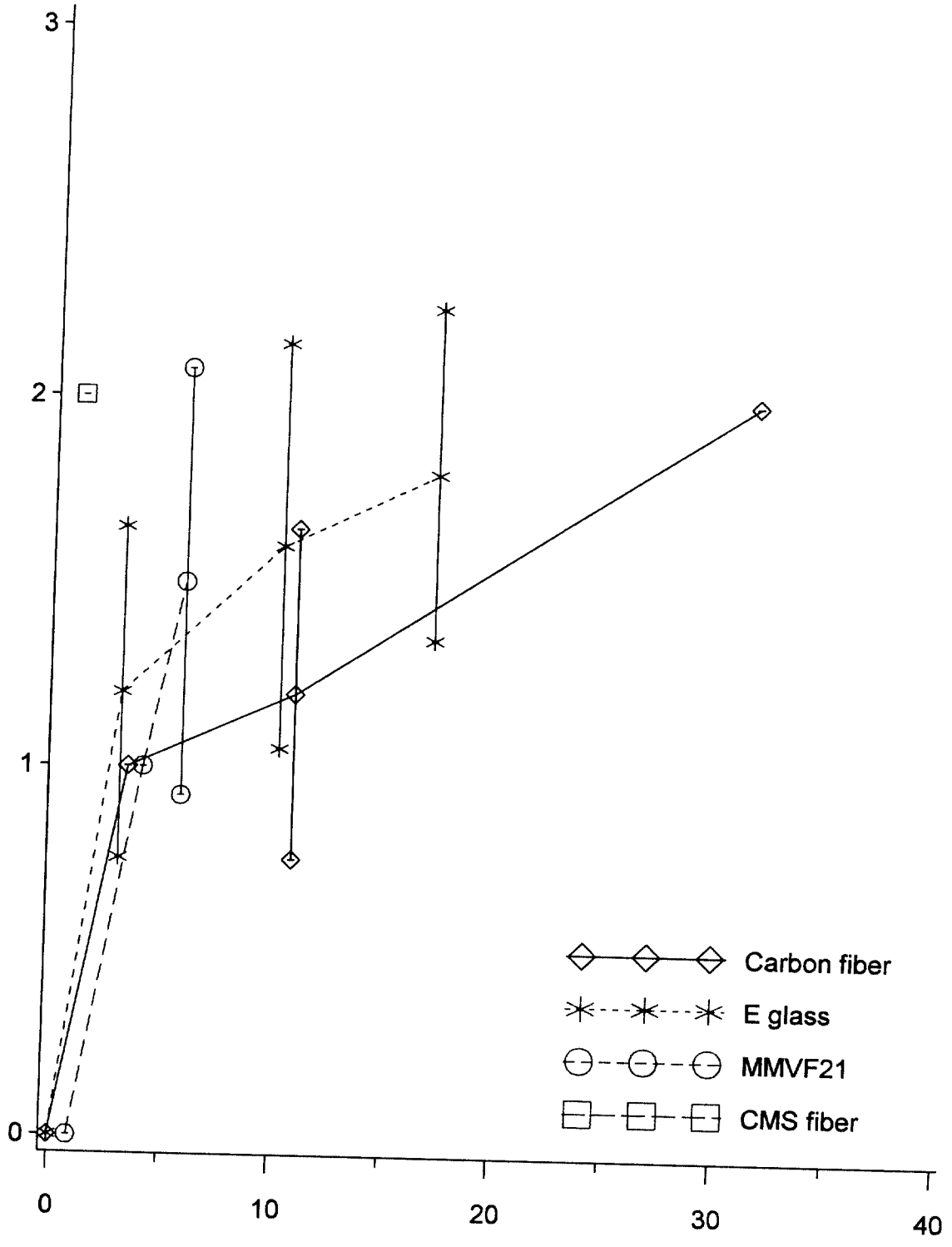
Fibers (L>20um) per lung x 10E6

EPS grade at 8 Weeks postexposure



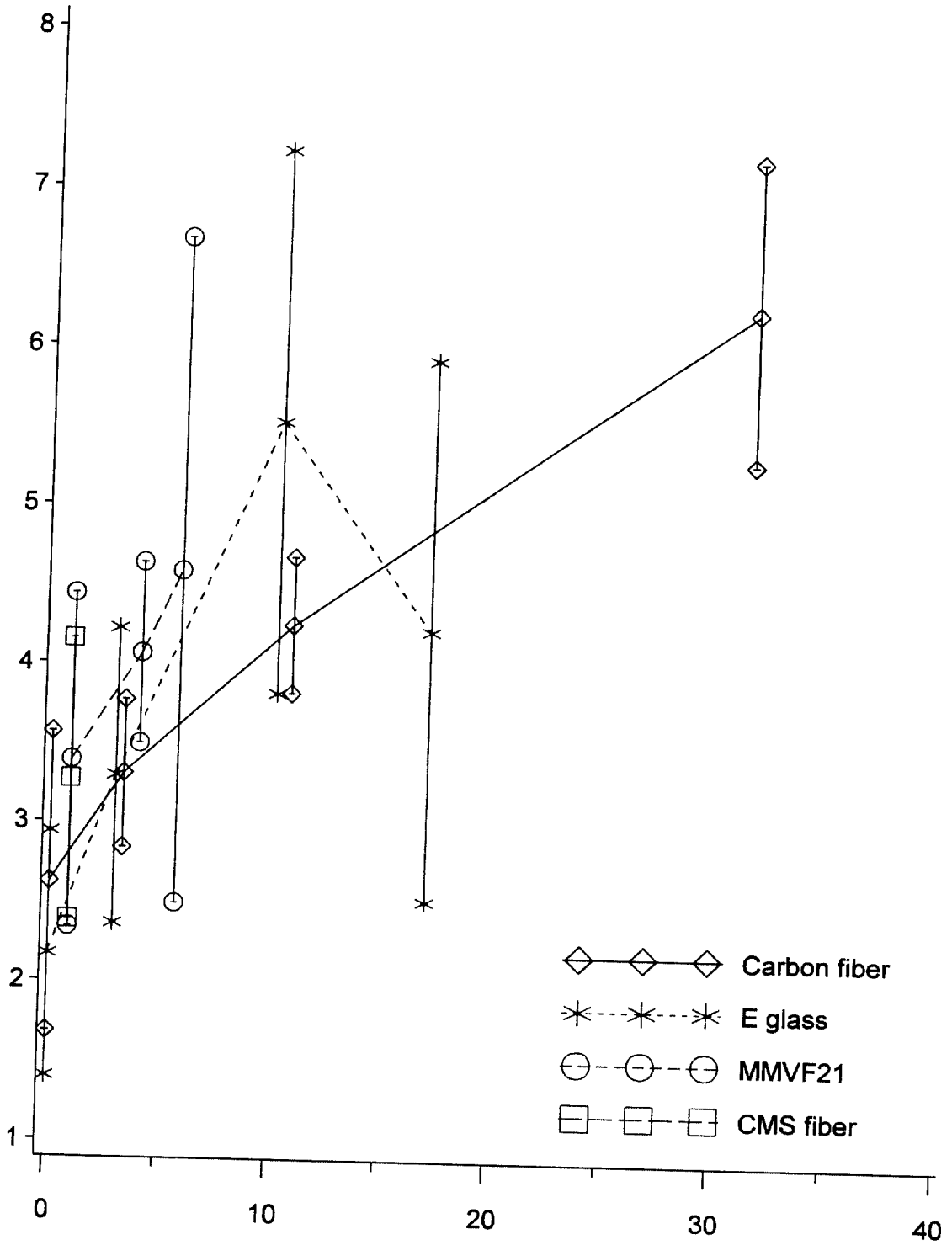
Fibers (L>20um) per lung x 10E6

EPS grade at 15 Weeks postexposure



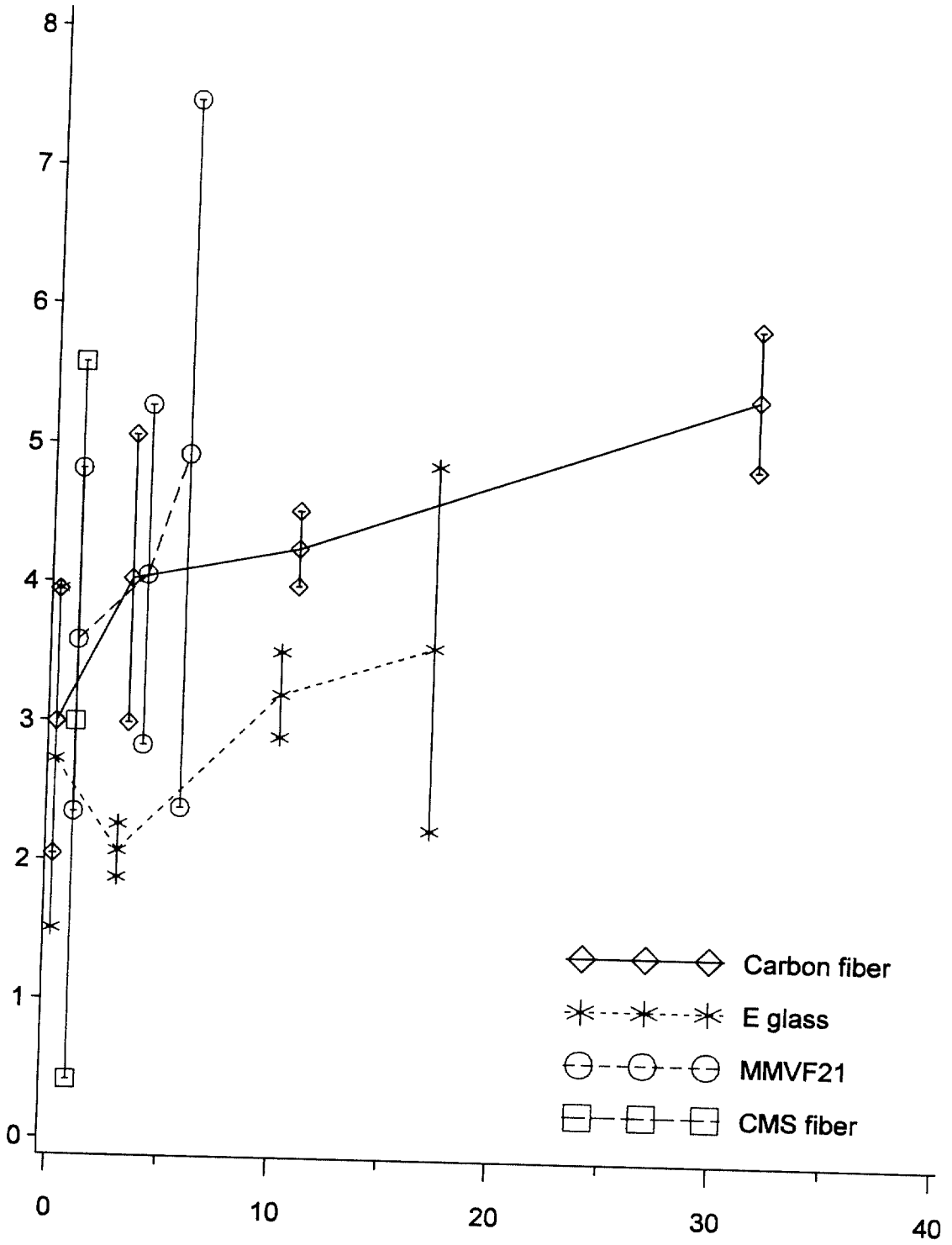
Fibers (L>20um) per lung x 10E6

Parenchymal cells [Labelling index %] at 1 Week postexposure

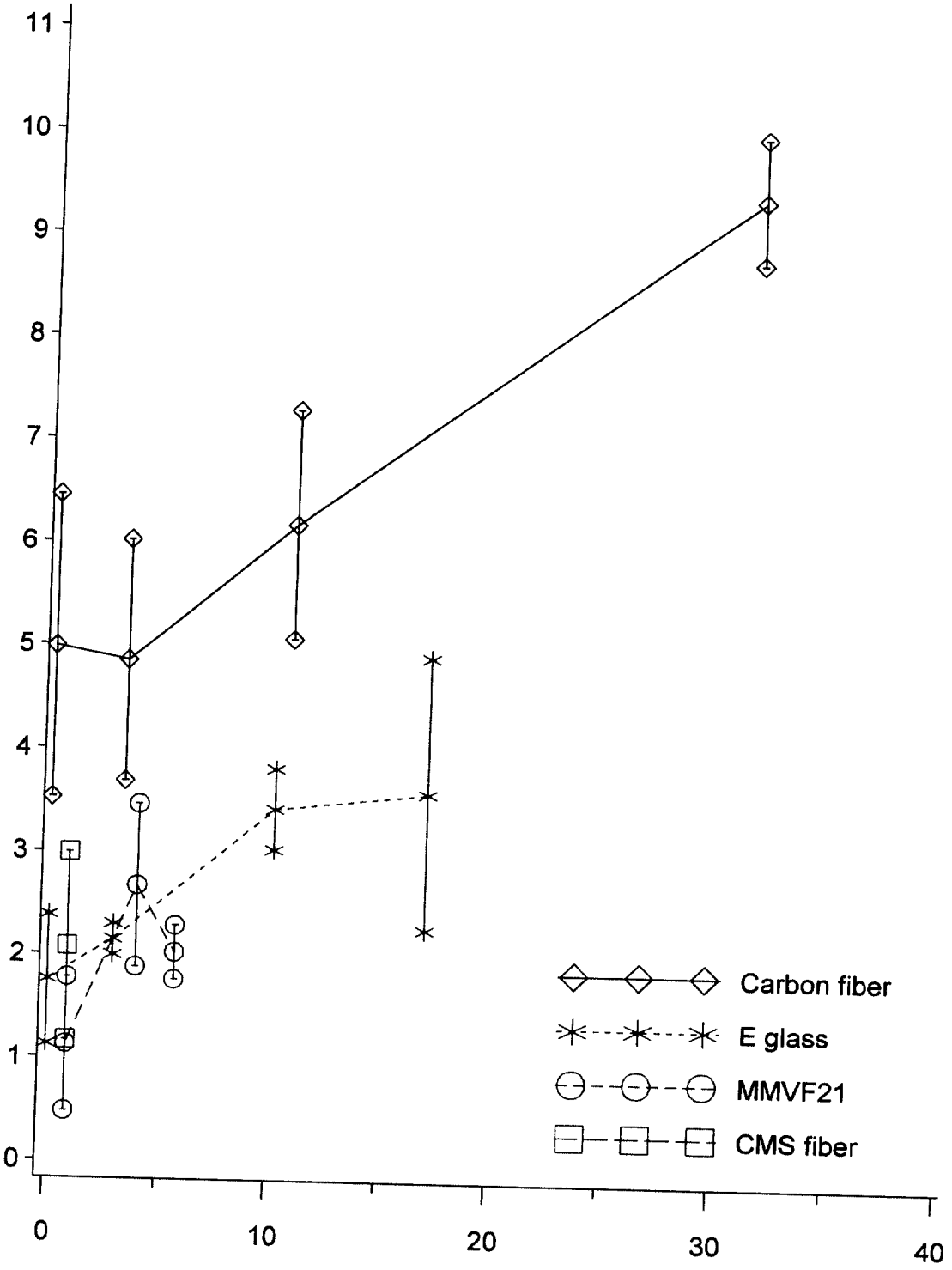


Fibers (L>20um) per lung x 10E6

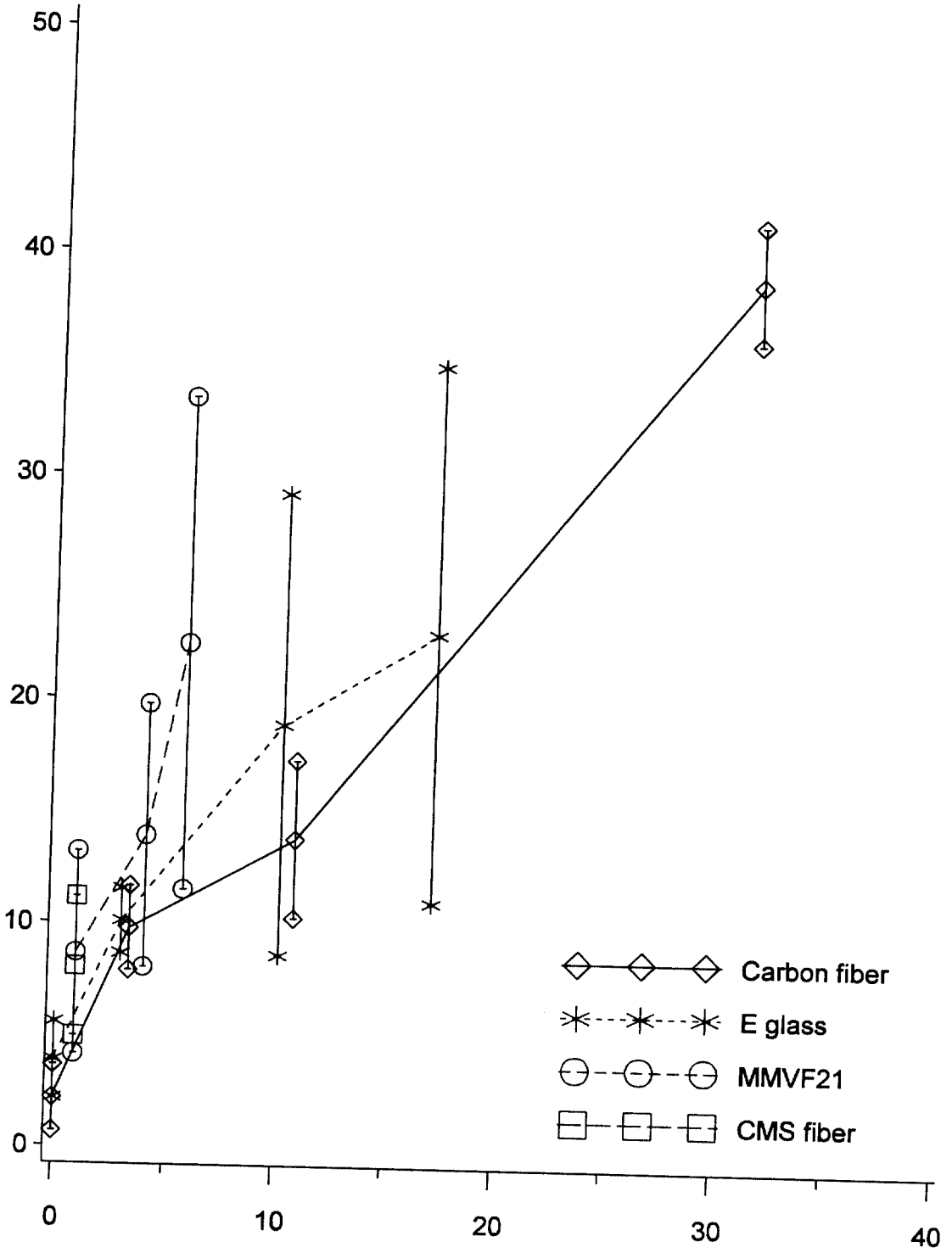
Parenchymal cells [Labelling index %] at 8 Weeks postexposure



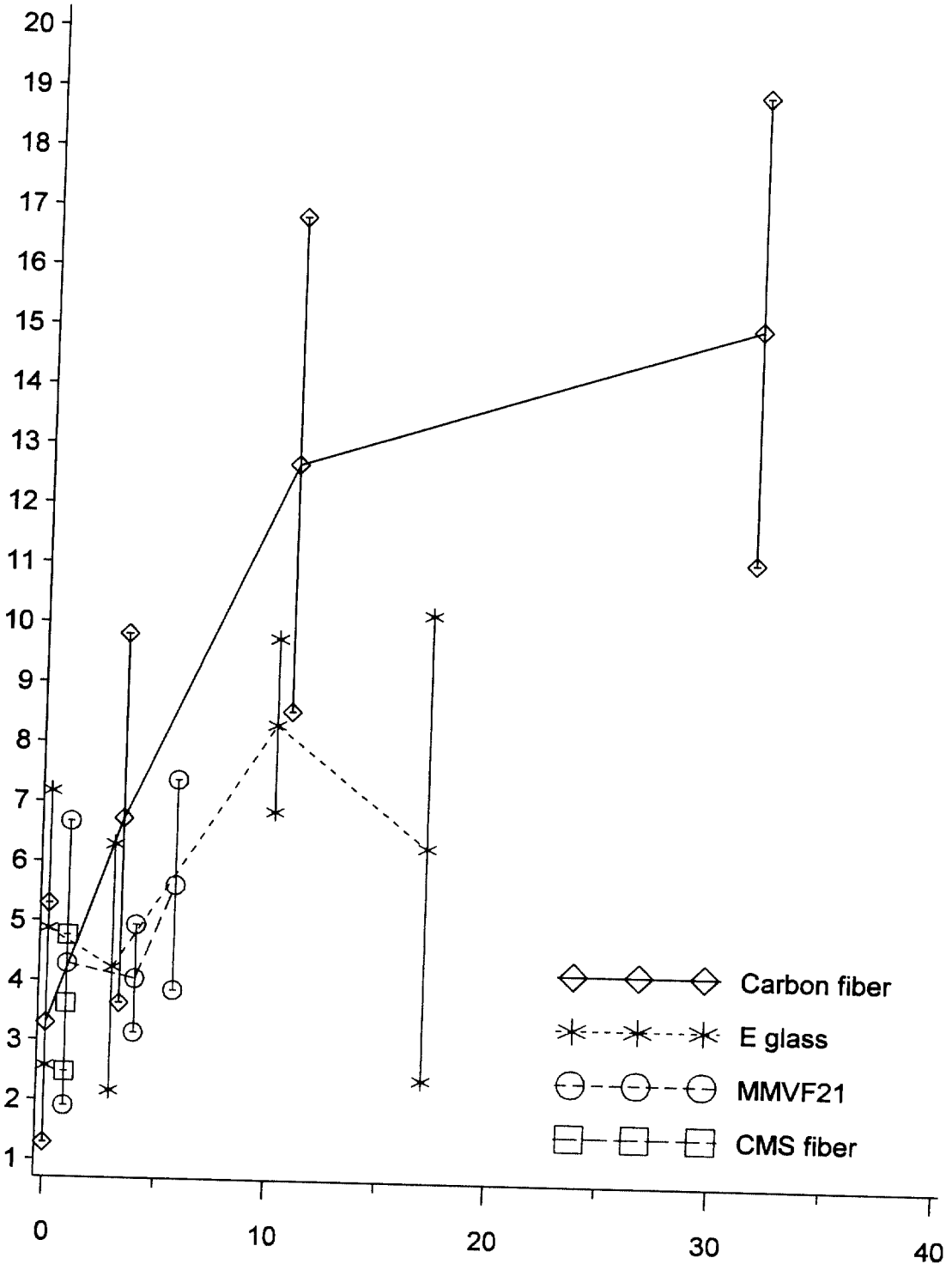
Parenchymal cells [Labelling index %] at 15 Weeks postexposure



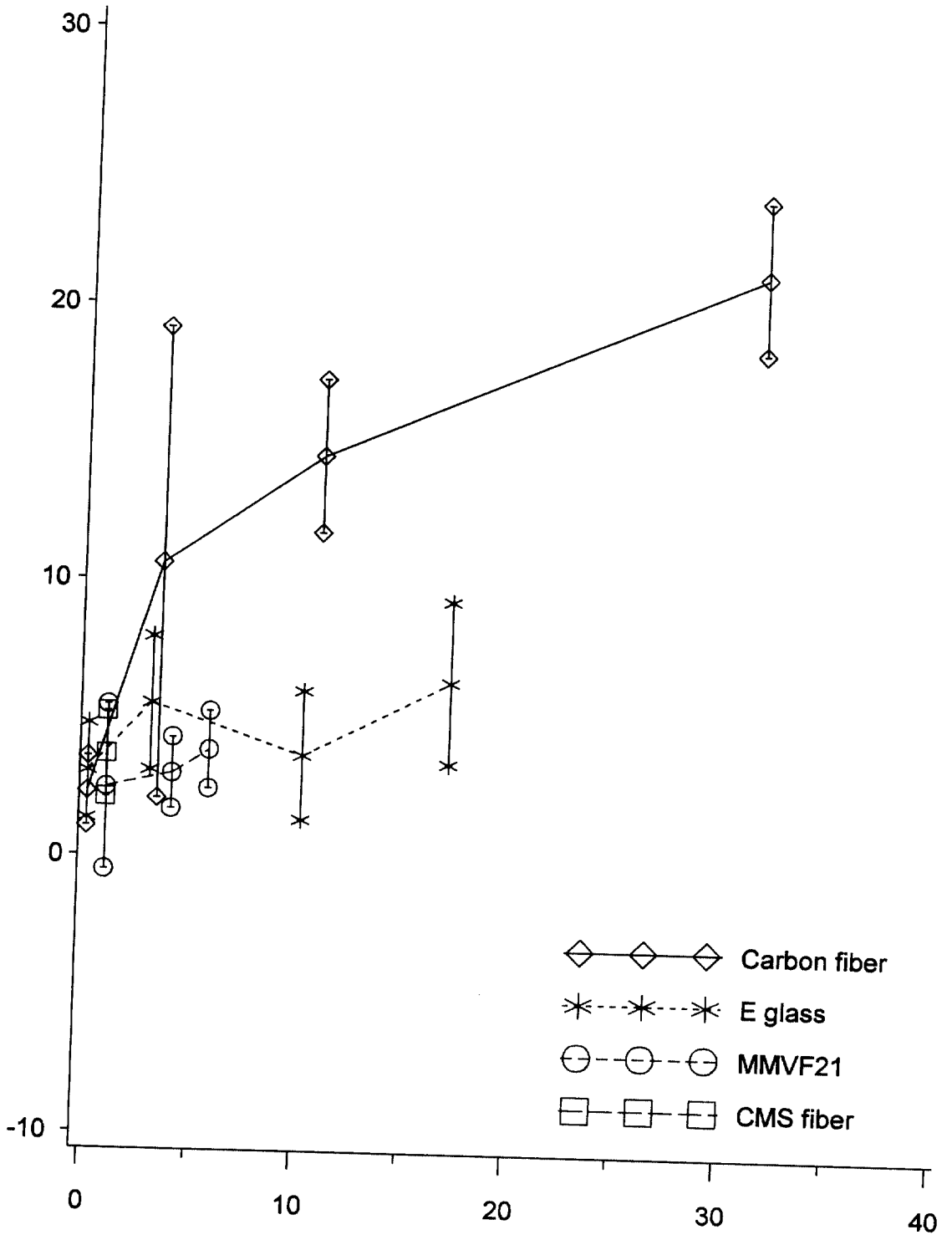
Terminal bronchioles [labelled cells/mm] at 1 Week postexposure



Terminal bronchioles [labelled cells/mm] at 8 Weeks postexposure



Terminal bronchioles [labelled cells/mm] at 15 Weeks postexposure



Appendix 11 Comparison of effects on cell proliferation induced by carbon fibers with those of E-glass, MMVF21 and CMS in a previous Fraunhofer ITEM study

Group	Unit length labelling index [%] of terminal bronchiolar epithelium at postexposure [Normalized to Percent of controls]								
	1 Week			8 Weeks			15 Weeks		
	Mean	SD	N	Mean	SD	N	Mean	SD	N
Control	100.00	68.82	5	100.00	60.92	5	100.00	54.54	5
Carbon fiber low	456.47	87.86	5	204.47	93.86	5	461.86	371.09	5
Carbon fiber med	644.46	163.94	5	386.16	125.99	5	633.79	120.69	5
Carbon fiber high	1816.06	123.27	5	459.28	119.15	5	928.94	120.37	5
Control	100.00	44.18	5	100.00	47.24	4	100.00	56.29	4
E-glass low	262.28	37.39	5	86.89	42.38	5	181.55	79.59	5
E-glass med	491.14	267.09	5	-- 170.93	29.62	4	--- 121.46	76.64	5
E-glass high	598.19	310.82	4	129.71	80.04	5	211.50	96.98	5
MMVF21 low	224.83	117.73	5	88.02	48.90	5	80.75	98.18	5
MMVF21 med	361.56	152.36	5	83.12	18.56	5	-- 98.54	42.39	5
MMVF21 high	584.58	284.99	5	115.50	36.14	5	126.75	45.99	4
CMS high	208.73	81.01	4	74.27	23.50	5	120.04	50.89	5
Group	Labelling index of lung parenchymal cells at postexposure [Normalized to Percent of controls]								
	1 Week			8 Weeks			15 Weeks		
	Mean	SD	N	Mean	SD	N	Mean	SD	N
Control	100.00	35.90	5	100.00	31.77	5	100.00	29.34	5
Carbon fiber low	126.08	17.67	5	134.55	34.57	5	97.41	23.37	5
Carbon fiber med	162.13	16.37	5	142.39	9.08	5	124.15	22.24	5
Carbon fiber high	238.80	36.35	5	180.22	16.86	5	188.80	12.26	5
Control	100.00	35.47	5	100.00	44.76	4	100.00	35.72	4
E-glass low	151.83	42.79	5	75.97	7.04	5	122.52	8.60	5
E-glass med	254.75	78.62	5	117.74	11.20	4	195.40	22.36	5
E-glass high	194.81	78.35	4	130.61	48.00	5	204.96	75.03	5
MMVF21 low	156.26	48.35	5	131.27	45.18	5	64.19	36.99	5
MMVF21 med	187.52	26.27	5	148.65	44.71	5	152.41	44.94	5
MMVF21 high	211.51	96.32	5	180.64	93.21	5	115.67	14.87	4
CMS high	150.74	40.74	4	109.83	94.51	5	118.32	51.92	5

Comparison of E-glass, MMVF21 and CMS to group of carbon fiber with similar lung burden (which means: E glass med - Carbon fiber med; E glass low - Carbon fiber low; MMVF21 med - Carbon fiber low)

Statistics: Tukey test: higher effects + P<=5%; ++ P<=1%; +++ P<=0.1%
lower effects - P<=5%; -- P<=1%; --- P<=0.1%

Appendix 11 (cont.) Comparison of effects on cell proliferation induced by carbon fibers with those of E-glass, MMVF21 and CMS in a previous Fraunhofer ITEM study

Group	Unit length labelling index [%] of pleural cells at postexposure [Normalized to Percent of controls]								
	1 Week			8 Weeks			15 Weeks		
	Mean	SD	N	Mean	SD	N	Mean	SD	N
Control	100.00	52.51	5	100.00	106.46	5	100.00	36.54	5
Carbon fiber low	111.55	58.23	5	212.32	46.07	5	149.05	78.64	5
Carbon fiber med	153.46	37.33	5	612.08	880.21	5	324.68	300.59	5
Carbon fiber high	483.57	384.26	5	210.72	164.73	5	186.48	118.37	5
Control	100.00	44.55	5	100.00	22.43	4	100.00	53.03	4
E-glass low	115.15	38.03	5	96.30	52.05	5	168.62	60.39	5
E-glass med	126.52	28.55	5	201.85	75.45	4	212.92	113.19	5
E-glass high	115.53	77.04	4	137.78	53.82	5	183.38	88.37	5
MMVF21 low	89.39	31.07	5	107.41	57.44	5	98.46	35.62	5
MMVF21 med	143.18	15.01	5	110.37	25.98	5	113.23	43.21	5
MMVF21 high	215.15	118.95	5	126.67	30.92	5	241.54	231.81	4
CMS high	120.27	45.07	4	75.56	30.04	5	141.54	47.67	5

Comparison of E-glass, MMVF21 and CMS to group of carbon fiber with similar lung burden (which means: E glass med - Carbon fiber med; E glass low - Carbon fiber low; MMVF21 med - Carbon fiber low)

Statistics: Tukey test: higher effects + P<=5%; ++ P<=1%; +++ P<=0.1%
lower effects - P<=5%; - - P<=1%; - - - P<=0.1%

RCC STUDY NUMBER 801314

CARBON FIBRE

3-MONTH BIOPERSISTENCE INHALATION STUDY IN THE RAT

Authors Paul A. Smith and U. Teichert

Test Facilities

RCC Ltd
Wölferstrasse 4, CH-4414 Füllinsdorf,
Switzerland and
Zelgliweg 1, CH-4452 Itingen, Switzerland

GSA Gesellschaft für Schadstoffmessung
und Auftragsanalytik GmbH (GSA)
Gut Vellbrüggen
D-41469 Neuss-Norf, Germany

REPORT

RCC

1 CONFIDENTIALITY STATEMENT

This report contains unpublished results and may not be cited or reproduced by any means in whole or in part without the permission of [redacted]

2 STATEMENT OF COMPLIANCE

RCC Study Number	801314
Test Item	CARBON FIBRE
Study Director	Paul A. Smith
Title	CARBON FIBRE: 3-Month Biopersistence Inhalation Study in the Rat

This study was performed in compliance with the Swiss Ordinance relating to Good Laboratory Practice, adopted February 2nd, 2000 [RS 813.016.5]. This Ordinance is based on the OECD Principles of Good Laboratory Practice, as revised in 1997 and adopted November 26th, 1997 by decision of the OECD Council [C(97)186/Final].

The present statement applies to the work performed at RCC Ltd, Wölferstrasse 4, CH-4414 Füllinsdorf & Zelgliweg 1, CH-4452 Itingen. Technical trials performed prior to the start of the study are not covered by GLP Regulations.

The GLP compliant performance of the preparation of lung samples was the responsibility of RCC Ltd, Environmental Chemistry & Pharmanalytics Division.

GSA is responsible for the part of the study performed at it's facility.

The composition or purity of the test item was not stated, this information is excluded from the statement of compliance.

There were no circumstances that may have affected the quality or integrity of the study.

Study Director Paul A. Smith

.....

Date: 28-SEP-2002

3 QUALITY ASSURANCE UNIT

RCC Ltd, Toxicology Division
CH-4452 Itingen, Switzerland

STATEMENT

RCC Study Number	801314
Test Item	CARBON FIBRE
Study Director	Paul A. Smith
Title	CARBON FIBRE: 3-Month Biopersistence Inhalation in the Rat

The general facilities and activities are inspected periodically and the results are reported to the responsible person and the management.

Study procedures with the exception of the pre-tests were periodically inspected. The study plan and this report were audited by the Quality Assurance Unit. The dates are given below:

Dates and type of QAU Inspections / Audits		Dates of Reports to the Study Director and to Management
02-AUG-2001	Study plan	02-AUG-2001
15-AUG-2001	Study based (test system, test item, treatment, observations, raw data)	15-AUG-2001
17-AUG-2001	Study based (test item, raw data)	17-AUG-2001
22-AUG-2001	Study based (necropsy, raw data)	22-AUG-2001
08-OCT-2001	Study based (test system, observations, raw data)	08-OCT-2001
19-NOV-2001	Study based (necropsy)	19-NOV-2001
27 to 29-AUG-2002	Report	29-AUG-2002

This statement also confirms that this final report reflects the raw data.

Quality Assurance

Th. Frei

.....

Date: 27-AUG-2002

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FIGURES

SUMMARY TABLES

TABLES OF INDIVIDUAL DATA

ATTACHMENT 1

ATTACHMENT 2

2 PREFACE

2.1 GENERAL

Title	CARBON FIBRE: 3-Month Biopersistence Inhalation Study in the Rat

[redacted]

Test Facilities	Study Conduct, Inhalation, In-Life Observation and Necropsy RCC Ltd, Toxicology Division (RCC TOX) Wölferstrasse 4, CH-4414 Füllinsdorf, Switzerland and Zelgliweg 1, CH-4452 Itingen, Switzerland Preparation of the Lung Samples RCC Ltd, Environmental Chemistry and Pharmanalytics Division (RCC ECP) Zelgliweg 1, CH-4452 Itingen, Switzerland Investigations and Analyses of Fibres GSA Gesellschaft für Schadstoffmessung und Auftragsanalytik GmbH (GSA) Gut Vellbrüggen, D-41469 Neuss-Norf, Germany

2.2 RESPONSIBILITIES

Study Director	Paul A. Smith (RCC TOX)
Study Co-ordinator	H. Huber (RCC TOX)
Technical Coordinator	H. Fleissner (RCC TOX)
Necropsy	Dr. K. Weber (RCC TOX)
Principal Investigator Preparation of the lung samples	Dr. R. Müller-Käfer (RCC ECP)
Principal Investigator Evaluation of the samples	U. Teichert (GSA)
Head of RCC Quality Assurance	I. Wüthrich

4.3 SCHEDULE

Delivery (Experimental Starting Date)	03-AUG-2001
Acclimatisation	03 to 14-AUG-2001
Exposure	15 to 19-AUG-2001
Sacrifice at the 1-day time point	20-AUG-2001
Sacrifice at the 3-day time point	22-AUG-2001
Sacrifice at the 15-day time point	03-SEP-2001
Sacrifice at the 29-day time point	17-SEP-2001
Sacrifice at the 92-day time point Experimental completion date	19-NOV-2001

Final Report
Study Completion Date 27-SEP-2002

4.4 ARCHIVING

RCC Ltd (CH-4452 Itingen, Switzerland) will retain the study plan, raw data, a sample of test item(s), specimens and the final report of the present study for at least ten years. No data will be discarded without the Sponsor's consent.

Excluded are the study material and samples forwarded by RCC to GSA, who will archive the material under their responsibility.

4.5 METHOD GUIDELINES

The study procedures in this study were based on the EU Guidelines: Biopersistence of Fibres. Short Term Exposure by Inhalation, ECB/TM/26 rev. 7, 1998.

4.6 SUMMARY OF DEVIATIONS

The identification of the 14-day and the 30-day sacrifices were corrected and adapted to 15-day and 29-day sacrifices, respectively.

The description of the generation system was adapted.

Clinical signs and body weights were recorded more often than stated in the study plan.

In the first amendment to study plan, no management signature was included. Management documented their approval of the contents later, on a separate document.

The purity of the test item was not added by amendment.

The method for chemical digestion of the freeze-dried lungs was not specified by amendment to study plan.

No statistical analysis of the non-fibre particles was performed.

RCC STUDY NUMBER 801314
CARBON FIBRE

SIGNATURE PAGE

STUDY DIRECTOR — Paul A. Smith

.....
Date: 24-SEP-2002

MANAGEMENT — S. Corney (for)

.....
Date: 27- Sept.- 2002

5 SUMMARY

The purpose of this study was to assess the in-vivo pulmonary biopersistence of inhaled fibrous and non-fibrous particles in the rat following repeated inhalation exposure.

Laboratory rats were randomly assigned to one negative control group and to one group exposed to the test item, Carbon Fibre. The animals were exposed to filtered air (negative control) or to well characterised fibres which were sized to be rat respirable using a flow past, nose only exposure system. In the fibre-exposed group, the concentration was targeted to 15 mg/m^3 if technically feasible. The achieved concentration was 16 mg/m^3. The rats were exposed for five consecutive days, 6 hours per day, with a subsequent non-exposure period. In the control and in the fibre-exposed groups, rats were allocated to sub-groups of 5 and 7 animals respectively and sacrificed at 1 day, 3 days, 15 days, 29 days and 92 days.

For each of the sacrifice time-points the lung burden was determined by suitable extraction and measurement methods. Evaluation included counting of the number of fibres and particles in the lungs and characterisation of the fibres by bivariate analysis of diameter and length.

5.1 ANIMAL DATA

The in-life data recorded during the study - mortality, clinical signs and body weights - gave no indications of fibre-related findings.

Macroscopic findings at the 1 and 3-day sacrifice time-points indicated a minor, acute response to inhalation of a particulate material. Increased lung weights at the 29 and 92-day sacrifice time-points were observed, but it is not clear as to whether these were treatment related.

5.2 LUNG BURDEN DATA

The main results of lung burden analyses by SEM performed at GSA are summarised below. Details are presented in Attachment 2.

Group 2, CARBON FIBRE						
time point	1 day	3 days	15 days	29 days	92 days	T ½ days
FIBRE NUMBER (10^6 / lung)						
Total fibres/lung	83.858	82.122	61.764	53.500	25.378	45.13s
WHO fibres/lung	51.676	48.074	37.825	33.259	15.705	50.36s
Fibres/lung < 5 µm	30.676	32.583	22.716	18.932	9.195	47.14d
Fibres/lung 5-20 µm	50.133	46.599	36.716	32.377	14.450	46.10s
Fibres/lung > 20 µm	3.049	2.940	2.332	2.191	1.734**	99.86s
Total length of fibres/lung (m)	637.3	603.1	473.3	417.4	213.5	49.82s
Non-fibre particles	42.148	55.080	40.861	36.714	17.961	---
n=	6*	7	7	7	7	

* Animal 22 was excluded because the value of the total fibre length (m), and the total number of fibres per lung, differed from the average value of all animals evaluated at the same time-point by more that two times the value of the standard deviation.

** The number of fibres > 20μm was statistically significantly lower ($p < 0.01$) on day 92 compared with day 1.

s Single exponential

d double exponential (weighted value)

6 PURPOSE

The purpose of this study was to assess the in-vivo pulmonary biopersistence of inhaled fibrous and non-fibrous particles in the rat following repeated inhalation exposure for 5 days.

Laboratory rats were randomly assigned to one negative control group and to one group exposed to the test item, Carbon Fibre. The animals were exposed to filtered air (negative control) or to well characterised fibres which were sized to be rat respirable using a flow past, nose only exposure system. In the fibre-exposed group, the concentration was targeted to 15 mg/m^3. The rats were exposed for five consecutive days, 6 hours per day, with a subsequent non-exposure period. In the control and in the fibre-exposed groups, rats were allocated to sub-groups of 5 and 7 animals respectively, planned for sacrifice at 1 day, 3 days, 15 days, 29 days and 92 days.

The lung burden was determined at the different sacrifice time-points by suitable extraction and measurement methods. Evaluation included counting of the number of fibres and particles in the lungs and characterisation of the fibres by bivariate analysis of diameter and length.

7 MATERIALS AND METHODS

7.1 TEST SYSTEM

Test System	Male Wistar Rat, HanBrl:WIST (SPF)
Rationale for Selection	Routinely used as a standard for studies with natural and/or man-made fibres
Source	RCC Ltd, Biotechnology and Animal Breeding Division (RCC BAB) Wölferstrasse 4, CH-4414 Füllinsdorf, Switzerland
Number of Groups	2
Number of Animals per Group	Air control: 15 rats Treated group: 35 rats
Total Number of Animals in the study	55 (including 5 reserve animals)
Body Weight at Delivery	183.8 - 208.8 grams
Age at Delivery	9 weeks
Acclimatisation	From 03 to 14-AUG-2001. A clinical health examination was performed on all animals shortly after delivery. There were no visible findings of illness and all animals entered the acclimatisation phase. No substitution of study animals with reserve animals was necessary prior to the start of the exposure and all reserve animals were sacrificed on the first day of treatment, before treatment start.
Randomisation	On the day of delivery (03-AUG-2001), all animals were assigned to the different groups using a random sampling procedure stratified by body weight.
Identification	By ear tattoo. Housing cages were identified with the appropriate animal numbers. During the first seven days of the acclimatisation phase, the animals were identified by cage numbers and code on the tail.

Habituation to restraint The rats were accustomed to the restraint tubes during the acclimatisation phase by maintaining them in tubes for 5 daily periods of approximately 30 minutes, 1, 2, 4 and 5 hours.

7.2 ALLOCATION

Sacrifice Time Points	Group 1, Air Control	Group 2, Carbon Fibre
1 Day	1 – 5	16 - 22
3 Days		23 - 29
15 Days		30 - 36
29 Days	6 – 10	37 - 43
92 Days	11 – 15	44 - 50
Reserve animals	51 - 55	

7.3 HUSBANDRY

Room Numbers

Acclimatisation, treatment, first 6 days of recovery Room 3.16 (RCC, Füllinsdorf)
From day 7 of recovery until the end of the study Room 3.06 (RCC, Füllinsdorf)

Laboratory Conditions

The study was conducted under Optimal Hygienic Conditions (OHC) behind a barrier system. The conditioned air was supplied with 10-15 air changes per hour, a monitored environment with target range for temperature of 22 ± 3°C and for relative humidity of 30 - 70%, a period of 12 hours of artificial fluorescent light and a 12 hour dark period. Music was played during the major part of the light period.

Accommodation

Animals were housed in groups of 5, maximally, in Makrolon® Type IV cages with wire mesh tops and standard, dust-free softwood bedding ("Lignocel", Schill AG, CH-4132 Muttenz, Switzerland) during the major part of the acclimatisation and post-exposure phases. From day 8 of acclimatisation until day 3 of recovery, the animals were housed in sealed, negative pressure chambers used for group isolation. Bedding and cages were autoclaved before use. Tubes and plastic equipment were exposed to formaldehyde vapour before use.

Diet

Pelleted standard Kliba 3433 rat maintenance diet, batches no. 71/01 and 73/01 (Provimi Kliba AG, CH-4303 Kaiseraugst) *ad libitum*. Results of analysis for contaminants with acceptability limits are included in this report (Attachment 1, pp. 70 to 73).

Water

Community tap water from Füllinsdorf, chlorinated to approximately 0.5 ppm, *ad libitum*. Results of bacteriological, chemical and contaminant analysis with acceptability limits are included in this report (Attachment 1, pp. 74 to 76).

None of the contaminants present in the water and diet were expected to interfere with the study.

7.4 TEST ITEM

Identification / Test Item Name	CARBON FIBRE
Batch Number	P133-250401
Purity	Not stated
Expiry Date / Stability	December 2003
Instructions for Storage	In the original container at room temperature (19-25°C) protected from sunlight and moisture
Safety precautions	During the whole study, the use of a full face positive filtered (S3) air supply respirator, gloves and complete change of laboratory clothing was required.

Preparation of the Stock Fibres

The material produced by the Sponsor and submitted to RCC was referred to as *bulk material*. The material used for aerosolisation was referred to as *stock fibre* and was obtained from the bulk material.

The stock material was prepared at RCC and characterised at GSA. The dimensions of the stock material are summarised below:

	CARBON FIBRE	
	Diameter	**Length**
Geometric Mean (µm)	0.93	7.27
Geometric Mean F> 20 µm (µm)	1.54	27.25
% of fibres with L>20 µm	9.1	

7.5 TREATMENT

Route of administration	Inhalation by nose only exposure. This method of administration closely mimics that in humans and represents the natural route of uptake
Frequency and duration	6 hours/day, during 5 consecutive days

Target Exposure Concentrations

The exposure level was adjusted in accordance with the following criteria:

	Test Item	**Exposure Concentration**
Group 1	Filtered air	---
Group 2	Carbon Fibre	15 mg/m^3

The target exposure level was selected based upon the technical trials and in agreement with the Sponsor and the Monitoring Scientist.

The air control animals (Group 1) were treated with filtered air under the same conditions as animals exposed to fibres.

7.6 INHALATION EXPOSURE SYSTEM

Inhalation exposure was performed using a system similar to that originally described by Sachsse et al[1]. (1973, 1976). The animals are confined separately in Makrolon® tubes which are positioned radially around a flow-past, nose-only exposure chamber[2]. The design of this exposure system is based upon fluid dynamic modelling of the aerosol flow and is unique in comparison with conventional nose-only exposure systems in that it ensures a uniform test item distribution, provides constant stream of "fresh" test item to each animal, and precludes rebreathing from the exhaled air. The aerosol stream reaches the animal's nose through ports situated at different levels around the axis of the exposure chamber. Each level can be rotated allowing close observation of all animals without interruption of exposure.

7.6.1 GENERATION OF TEST AND REFERENCE TEST ITEMS

A fibre aerosol was produced using the RCC Fibre Aerosol Generation System. After passing through a cyclon (diameter, 5 cm) the fibre aerosol was delivered directly into the flow-past exposure chamber.

7.6.2 EXPOSURE SYSTEM MONITORING

Airflow Rate

Monitored daily by constant air pressure for the generation and by flow meter at the chamber extraction.

Oxygen Concentration

Monitored continuously during each exposure, at the position of the animal's snout, using an oxygen sensor (Pewatron AG, CH-8304 Wallisellen, Switzerland), connected to a datalogger.

Temperature and Relative Humidity

Monitored continuously in the air supply for the fibre generator, just prior to the fibre aerosol generation system, using a Rotronic Hygrometer, Serie I-200 (Rotronic AG, CH-8040 Zürich, Switzerland), connected to a datalogger. Although there were no specific targets set the purpose of monitoring these parameters was to avoid water condensation forming in the fibre generator, which could have occurred at high humidity and low temperatures.

---

[1] K. Sachsse, L. Ullmann, G. Voss and R. Hess: Measurements of inhalation toxicity of aerosols in small laboratory animals. In: Proceedings of the Europ. Soc. for the Study of Drug Toxicity, Vol. XV, pp. 239-251, Zürich, June 1973.

K. Sachsse, L. Ullmann, K. Zbinden: Toxikologische Prüfungen von Aerosolen im Tierexperiment, "Chemische Rundschau" 29 (1976), No. 38, Page 1.

[2] Cannon, W.C., Blanton E.F., and McDonald, K.E.:"The Flow-Past Chamber: An Improved Nose-Only

7.6.3 TEST ATMOSPHERE MONITORING

Technical Trials

Preliminary generation trials were performed at different gravimetric concentrations. The collected filters were forwarded to GSA for counting and sizing. On the basis of these results and in agreement with the Sponsor a gravimetric concentration of 15 mg/m^3 was chosen for exposure of the animals.

General

All fibre samples were collected on the appropriate filters in the vicinity of the animal's snout. On the inhalation tower the level of the port selected for inserting the filter holders was changed daily by one level occupied by the animals. Each day, filter holders for aerosol mass monitoring and for filter collection for other investigations was positioned at the same level.

One day during the exposure phase, simultaneous gravimetric samples were taken at all levels, for documenting that the uniformity for fibre concentrations at all levels was within ±15% of the average value.

Aerosol Mass Monitoring

In both groups, samples were collected daily on MILLIPORE membrane filters (Durapore HVLP, diameter 47 mm, pore size 0.45 μm). The duration of sampling was at least 5 hours. One sample was collected each day except on the second day when three samples, one at each level, were collected for the carbon fibre exposure chamber.

All collected samples were weighed for aerosol mass monitoring, and the results are presented in the report. All filters were discarded following the end of the exposure phase.

Short gravimetric samples were taken for daily fine adjustment of the concentrations. No specimens and no records were maintained. Additionally a Real Time Aerosol Sensor (RAS-2, MIE Inc., Bedford, MA 01730, USA) was used for technical monitoring during exposure time. This system allowed for the real time assessment of the exposure concentration. When calibrated and monitored, changes or malfunctions associated with the exposure system could be identified and corrected. Since this device was used for technical monitoring only, no data were reported, although some may be stored or may appear in the raw data.

Filter Collection for Other Investigations

In both groups, samples were collected daily on NUCLEPORE® filters (PC membrane, diameter 47 mm, pore size 0.2 μm). The duration of sampling was at least 5 hours and simultaneous with the gravimetric samples.

Two parallel samples were taken each day. One of them, scheduled for investigations, was sent to GSA. The other one was retained dry at RCC at disposal of the Sponsor, until issuing of the final report. Upon consultation with the Sponsor and unless otherwise requested, the retained samples were then discarded.

7.7 IN-LIFE OBSERVATIONS

Mortality

All animals were observed for mortality/moribundity once daily during the acclimatisation and recovery periods, and twice daily, once before and once after exposure, during the five days of the treatment phase.

Clinical Signs

Each animal had a detailed clinical observation for toxicological signs, including time of onset, intensity and duration:

- once on day 1 and once on day 8 of the acclimatisation phase
- twice daily (once before and once after the daily application) during the 5 treatment days
- once on the first day of recovery and at least once weekly thereafter.

Body Weights

Body weights were recorded:

- once on day 1 (used for randomisation) and once on day 8 of the acclimatisation phase
- on the day of first administration, prior to the administration start
- once on the first day of recovery and at least once weekly thereafter.

7.8 PATHOLOGY

Necropsy

All animals were necropsied for lung burden analysis according to the following schedule:

Sacrifice Time Points	Date	Group 1 Air Control	Group 2 Carbon Fibre
1 Day (24 ± 2 hrs)	20-AUG-2001	1 - 5	16 - 22
3 Days (72 ± 2 hrs)	22-AUG-2001	---	23 - 29
15 Days	03-SEP-2001	---	30 - 36
29 Days	17-SEP-2001	6 - 10	37 - 43
92 Days	19-NOV-2001	11 - 15	44 - 50

All animals were anaesthetised with an intraperitoneal injection of sodium pentobarbital (approximately 300 mg/kg bw.) and sacrificed by exsanguination.

The rats were submitted to complete macroscopical observation, including careful examination of the external surface of the body, thoracic and abdominal cavities and their contents. Gross findings at necropsy were recorded.

Organ Sampling

All action was taken to avoid contamination of the dissected specimen by fibres from the fur or deposited on dissecting instruments. Special care was taken to avoid inter-group contamination. During each necropsy session, air control animals were dissected first.

For each animal the following procedure was followed:

- **The lungs and trachea** (sectioned below the larynx) were removed with the attached mediastinal tissue. The mediastinal tissue containing the mediastinal lymph nodes was resected and immediately deep-frozen on dry ice and then stored at -20°C or below, at RCC for possible investigations prescribed by the Sponsor under separate contractual agreement. However, if not used, storage of these specimens ends by the time of submission of the final report. The remaining tissue/organs: lower half of the trachea, main stem bronchi and lungs were weighed (recorded as "lung weight").
- **The trachea and the main stem bronchi** down to the limit of the lung lobes were resected in one piece, weighed (recorded as "trachea"), individually inserted into plastic bags appropriately labelled and immediately deep-frozen on dry ice and then stored at -20°C or below, at RCC for possible investigations prescribed by the Sponsor under separate contractual agreement. However, if not used, storage of these specimens ends by the time of submission of the final report.
- **All lung lobes** were weighed together (recorded as "all lung lobes"), immediately deep-frozen on dry ice and forwarded (deep-frozen) to RCC ECP (attn: M. Kern). The lungs were freeze dried, weighed (recorded as "dry lung weight"), inserted into a plastic bag appropriately labelled, then stored in a desiccator at room temperature until further processing.

All freeze-dried lungs were chemically digested according to a method established at RCC and sent to GSA for lung burden investigations. The method comprised digesting the lungs in a heated acid mixture for a set duration followed by similar treatment with an oxidising agent and then vacuum filtering to trap the organic fibres on polycarbonate filters.

8 INVESTIGATIONS AT GSA

All specimens were sent from RCC TOX to GSA.

A signed and dated chain of custody form accompanied all samples sent to GSA. The chain of custody forms are archived at the GSA facilities. From the time of delivery at GSA, the specimens were under their responsibility. Preparation procedures as well as GLP regulation compliance are specified in the report submitted by GSA (Attachment 2).

The fibre-loaded Nuclepore filters scheduled for investigation by GSA were placed individually into 100 ml glass flasks with the appropriate care to preclude any spillage. The flasks containing the dry filters were sealed with a plastic top, adequately labelled and express mailed under ambient conditions to GSA. Each sample was labelled with the RCC study number, RCC filter number, group number and date of sampling.

The containers with the digested lung samples were express mailed under ambient conditions to GSA. Each sample was labelled with the RCC study number, animal number, group number and fibre identification, date of necropsy and name of tissue.

An overview of the number of samples investigated at GSA is presented below:

Group	Exposure Aerosol	
	F/ml	BVA
1 Air Control	5	---
2 Carbon Fibre	5	5

Group	Lung Burden: F/Lung and BVA				
	1 day	3 days	15 days	29 days	92 days
1 Air Control	5	---	---	5	5
2 Carbon Fibre	7	7	7	7	7

BVA = Bivariate analysis of fibre size distribution
F/ml = Fibre counting (expressed in fibre number per ml of suspension)
F/lung = Fibre per lung

9 STATISTICAL ANALYSIS

Analysis of weights

The following statistical methods were used to analyse body weight and organ weights:

- If the variables could be assumed to follow a normal distribution, the t-test comparing both groups was used.
- The Steel-test (many-to-one rank test) was applied when the data could not be assumed to follow a normal distribution.

Group means with standard deviations (S.D) were calculated for continuous data; for discrete data (scores), medians were calculated.

Analysis of lung burden data

The list of parameters is available in the report by GSA filed in Attachment 2 of the present report.

Analysis of Fibre Biopersistence in Lungs

The biopersistence of fibres in the rat lung was expressed by the half-time value ($T_{1/2}$), derived from the mathematical description of the fibre clearance:

The clearance was calculated for the following categories:

- total fibres
- WHO-fibres
- total fibres < 5 µm
- total fibres ≤ 5 µm and < 20 µm
- total fibres ≤ 20 µm

The clearance was essentially calculated by using the procedure described in the European regulations ECB/TM/27 rev. 7, Sections 2.5.2 and 2.5.3:

- the 100% value was fixed at day one after cessation of exposure
- a non-linear, single exponential regression was used to fit the data if the regression explained at least 80% of the variance: percent fibre remaining =
- otherwise a two-exponential was used:
- percent fibres remaining = a1*exp(-b1*days)+a2*exp(-b2*days)

The clearance half-time was calculated as follows:

- case of a single exponential: T1/2 = ln2/b
- case of a double exponential: summing the product of each half-time value by its coefficient ax.

All animals were used for evaluation, except if the value of the total fibre length (m), and the total number of fibres per lung, differed from the average value of all animals evaluated at the same time-point by more than two times the value of the standard deviation. In this case, the animal was removed from the statistical evaluation.

A SAS/STAT statistical analysis was also performed on the number of fibres longer than 20 µm at all time-points using the day 1 value as reference.

References

C.W. Dunnett: A Multiple Comparison Procedure for Comparing Several Treatments with a Control, J. Amer. Stat. Assoc. 50, 1096-1121 (1955).

R.G. Miller Simultaneous Statistical Inference, Springer Verlag, New York (1981).

SAS/STAT SAS/STAT User's Guide, Version 8, Cary, NC: SAS Institute Inc., 1999. The GLM Prodedure, 1465-1636.

10 DATA COMPILATION

Body weights, organ weights, mortality, clinical signs and macroscopical findings at necropsy were recorded on-line or on data sheets and then transferred into the Digital VAX computer system for compilation.

The fibre aerosol monitoring data were recorded on data sheets and manually entered into Microsoft Excel97 tables for calculations.

Individual values were rounded before printing. All derived values that appear in the tables represent the rounded results of calculations, which used the exact raw data value.

11 RESULTS

11.1 EXPOSURE CONDITIONS

During the daily 6-hour exposure period, rats were maintained in restraint tubes placed radially on inhalation towers, installed in Hazleton 2000-type chambers for group isolation. The humidity in the generator air was kept between 1.8 and 1.9% to avoid condensation of water in the fibre generator, and the temperature of the generator air remained between 21.2 and 21.9°C.

The airflow for group 1 remained at 32 litres/minute and the air pressure for group 2 at 0.3 bar, equating to 81 litres/minute. Each system comprised of 7 stages with 16 outlets/stage. For each group the appropriate number of outlets remained open to provide airflow of 1 litre/minute at each outlet. The oxygen concentration in the exposure system for groups 1 and 2 was between 20.8 and 21.8%.

The temperature and relative humidity in the animal room were recorded manually 3 times per exposure and remained stable between 21.4 and 22.0°C and 63.4 and 73.7% respectively.

11.2 AEROSOL DATA

Aerosol Concentrations

	Aerosol Mass Concentration (mg/m^3)		Mean Fibre Number (F/cm^3)			Mean Non-Fibrous Particle (nfp) (nfp/cm^3)
	Target	Achieved	Total	WHO	Fraction L > 20 μm	Total
Air control	0	0.012 ± 0.024	0.014	0	0	0.02
Carbon fibre	15	16.0 ± 1.48	1611	1046	92	888

mean = arithmetic mean
total = all objects with a 3:1 ratio (minimum) without any limits for length and diameter
WHO = fibre with a minimum in length of 5 μm and a maximum in diameter of 3 μm
fraction = number of fibre with length > 20 μm
nfp = any object with aspect ratio less than 3 was defined as a non-fibrous particle

The achieved gravimetric concentration was close to the target concentration.

Over the 5 days of inhalation exposure the variation of the carbon fibre aerosol concentration was approximately 9% demonstrating that the aerosol generation was reproducible. The variation of the carbon fibre aerosol concentration measured over the three stages of the inhalation chamber was less than 1% demonstrating that the rats attached to the different stages received the same aerosol concentration.

Aerosol Fibre Dimension

	Diameter	Length
Arithmetic Mean (μm) ± S.D.	1.04 ± 0.54	8.49 ± 7.19
Geometric Mean (μm) / G.S.D.	0.92 / 1.68	6.74 / 1.92
Median (μm)	1.0	6.4
Geometric Mean Fibres > 20 μm (μm)	1.15	28.72
% of fibres with L>20 μm	6%	

The data for samples taken during the exposure period were comparable with the dimensions of the stock material.

11.3 IN-LIFE OBSERVATIONS

Mortality data

There was no spontaneous mortality during the course of this study. All animals were sacrificed at the scheduled necropsy.

Clinical Signs

No clinical signs were observed during the course of this study, therefore no tables are included.

Body Weights

Figure, p. 32, Summary pp. 34 to 36, Individual data pp. 50 to 52.

Control animals and fibre-treated animals had similar mean body weights. No test item related effects were noted.

11.4 NECROPSY DATA

Organ Weights

Summary data: pp. 37 to 46; Individual data pp. 53 to 62.

After 29 and 92 days of recovery, lung weights including the mediastinum (absolute and relative to body weight) for group 2 (carbon fibre) were statistically significantly higher than air control. However, the weight of the lung lobes without mediastinum was only significantly increased on Day 92. It is not clear, therefore, as to whether this is treatment related since there are no increases in lung weight at the sacrifice time-points up to 29 days after the last exposure.

After 29 days of recovery absolute and relative tracheal weights for group 2 (carbon fibre) were statistically significantly higher than air control. In the absence of any statistical differences at any other time-point this finding was considered to be incidental.

Macroscopic Findings

Summary data: p. 47; Individual data pp. 63 to 68.

No macroscopical findings were noted in the control animals. Fibre-treated animals at the 1-day and the 3-day sacrifice time-points showed foci and/or not collapsed lungs. These findings were attributed to treatment since they were not present in control animals. Since there was a reduced number of animals affected at the 3-day sacrifice compared with the 1-day sacrifice and as there were no macroscopic findings at the later time-points, the observed findings were considered to reflect a minor, acute response to inhalation of particulate material.

11.5 LUNG BURDEN - CHARACTERISATION OF THE FIBRES

The table below summarises the main results for the fibre number and dimensions obtained at lung burden analysis at the successive sacrifice time points.

GSA describes the methods, the rules for fibre counting as well as the results of these analyses in the report, see Attachment 2, pp. 78 to 126. Figures of fibre clearance see pp. 26 to 31.

Group 2, CARBON FIBRE						
time point	1 day	3 days	15 days	29 days	92 days	T½ days
FIBRE NUMBER (10^6 / lung)						
Total fibres/lung	83.858	82.122	61.764	53.500	25.378	45.13s
WHO fibres/lung	51.676	48.074	37.825	33.259	15.705	50.36s
Fibres/lung < 5 µm	30.676	32.583	22.716	18.932	9.195	47.14d
Fibres/lung 5-20 µm	50.133	46.599	36.716	32.377	14.450	46.10s
Fibres/lung > 20 µm	3.049	2.940	2.332	2.191	1.734**	99.86s
Non-fibre particles	42.148	55.080	40.861	36.714	17.961	---
N=	6[1]	7	7	7	7	
DIAMETER						
Arithmetic Mean (µm)	0.96	0.96	0.99	0.98	0.99	
± S.D	0.44	0.44	0.46	0.45	0.45	
Geometric Mean (µm)	0.87	0.86	0.89	0.88	0.89	
S.D	1.59	1.61	1.60	1.59	1.59	
Median (µm)	0.9	0.9	0.9	0.9	0.9	
LENGTH						
Arithmetic Mean (µm)	7.60	7.34	7.66	7.80	8.41	
± S.D	5.47	5.34	5.67	5.68	6.67	
Geometric Mean (µm)	6.24	6.02	6.26	6.39	6.62	
± S.D	1.85	1.84	1.85	1.85	1.96	
Median (µm)	6.1	5.8	6.0	6.0	6.2	
Total length/lung (m)	637.3	603.1	473.3	417.4	213.5	49.82s
Particle/lung (10^6)	42.148	55.080	40.861	36.714	17.961	

** The number of fibres > 20µm was statistically significantly lower ($p < 0.01$) on day 92 compared with day 1.

s/d Single/double (weighted value) exponential

[1] Animal 22 was excluded because the value of the total fibre length (m), and the total number of fibres per lung, differed from the average value of all animals evaluated at the same time-point by more that two times the value of the standard deviation.

Assessment

- the overall clearance is not far above that of a biosoluble fibre
- considering the fibre size compartments, it is likely that macrophage clearance of short fibres (< 15 μm) is likely to be the most efficient mechanism
- clearance of the long fibres (> 20 μm) is less efficient. However, the decrease in the lung fibre burden (number of fibres > 20 μm at day 92 compared with day 1) was significant and likely to be caused by breakage of the long fibres into smaller ones which then are cleared by normal mechanical clearance processes (phagocytosis and removal by alveolar macrophages).

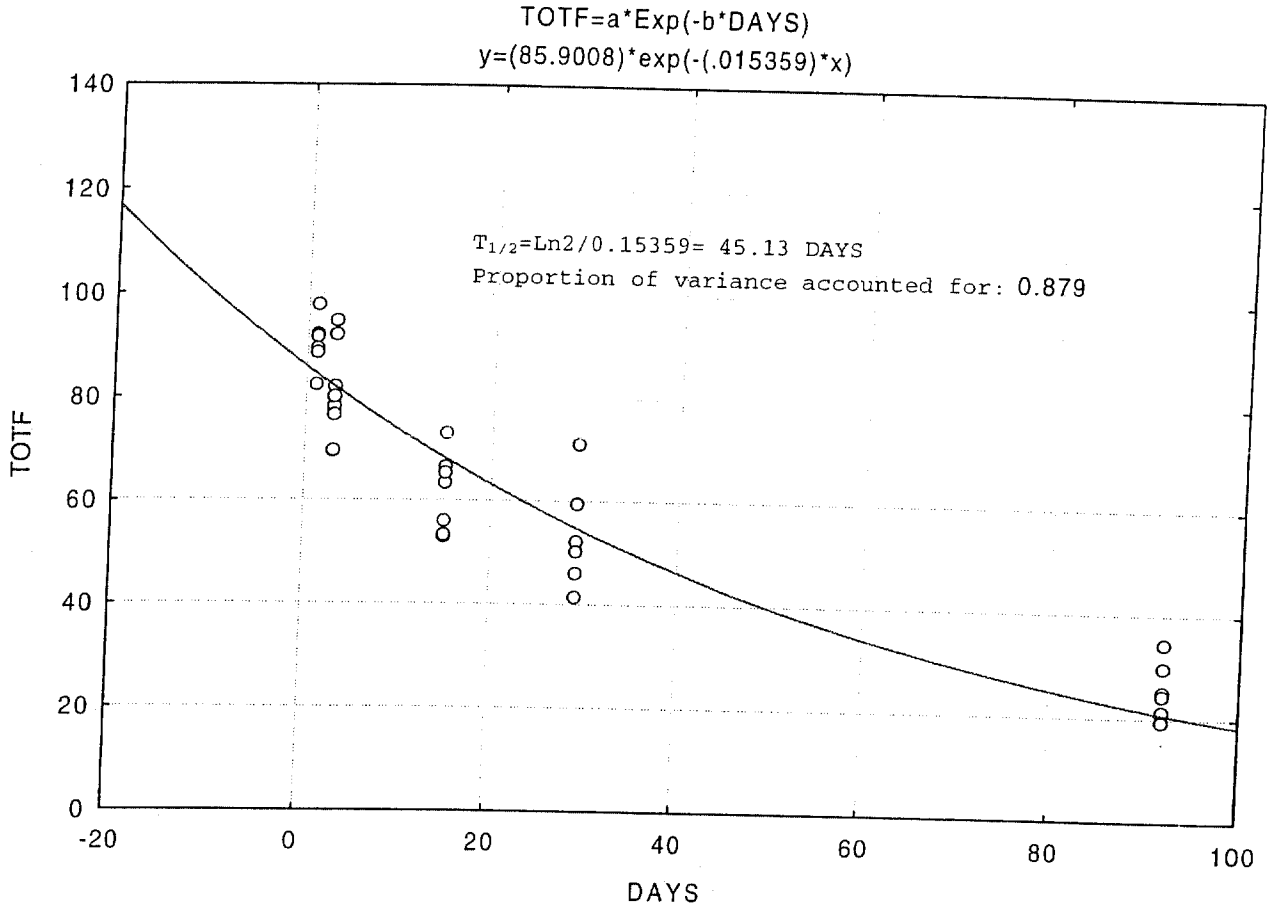
FIGURES

FIBRE CLEARANCE

BODY WEIGHTS

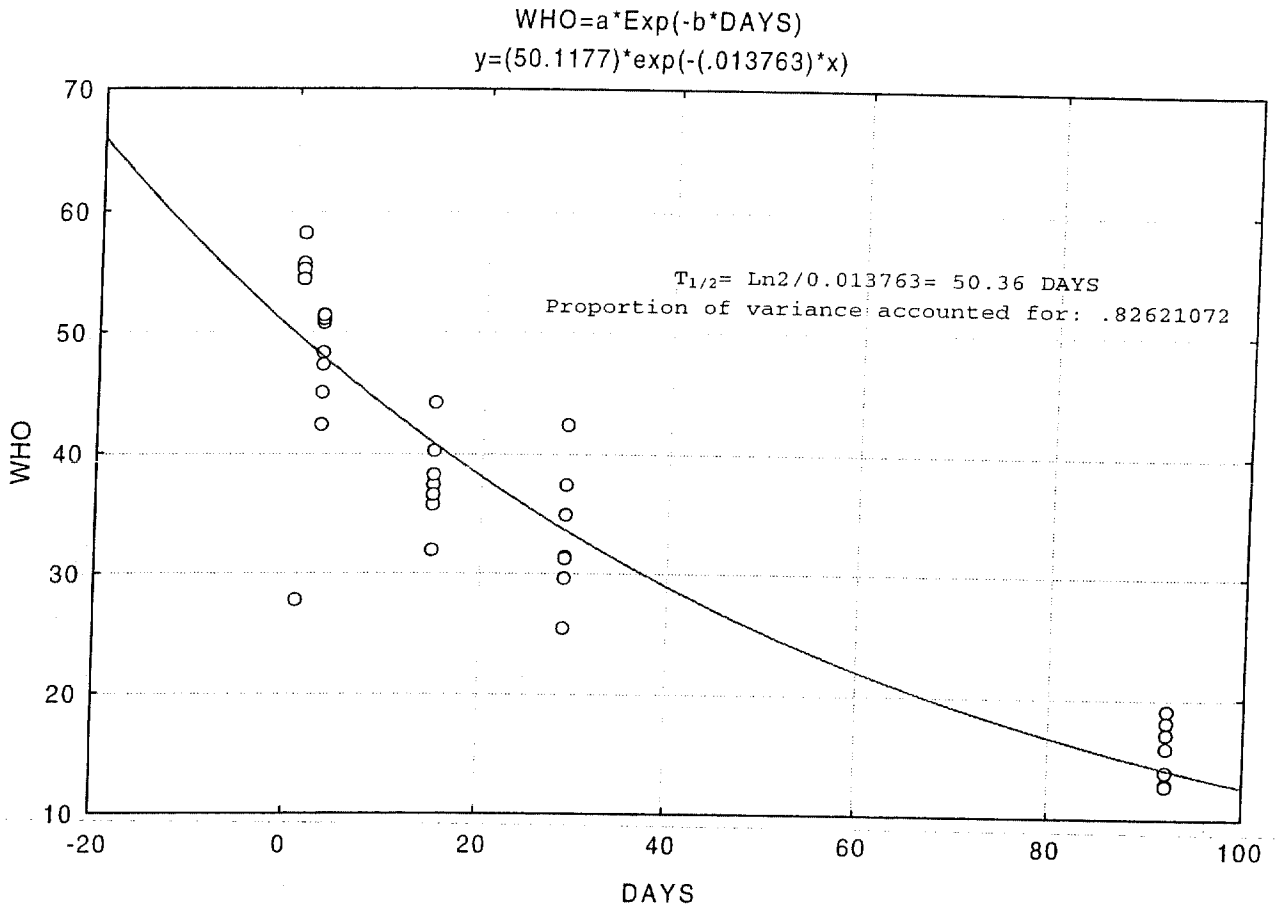
RCC STUDY NUMBER 801314
CARBON FIBRE

Total Number of Fibres (Mio/lung)



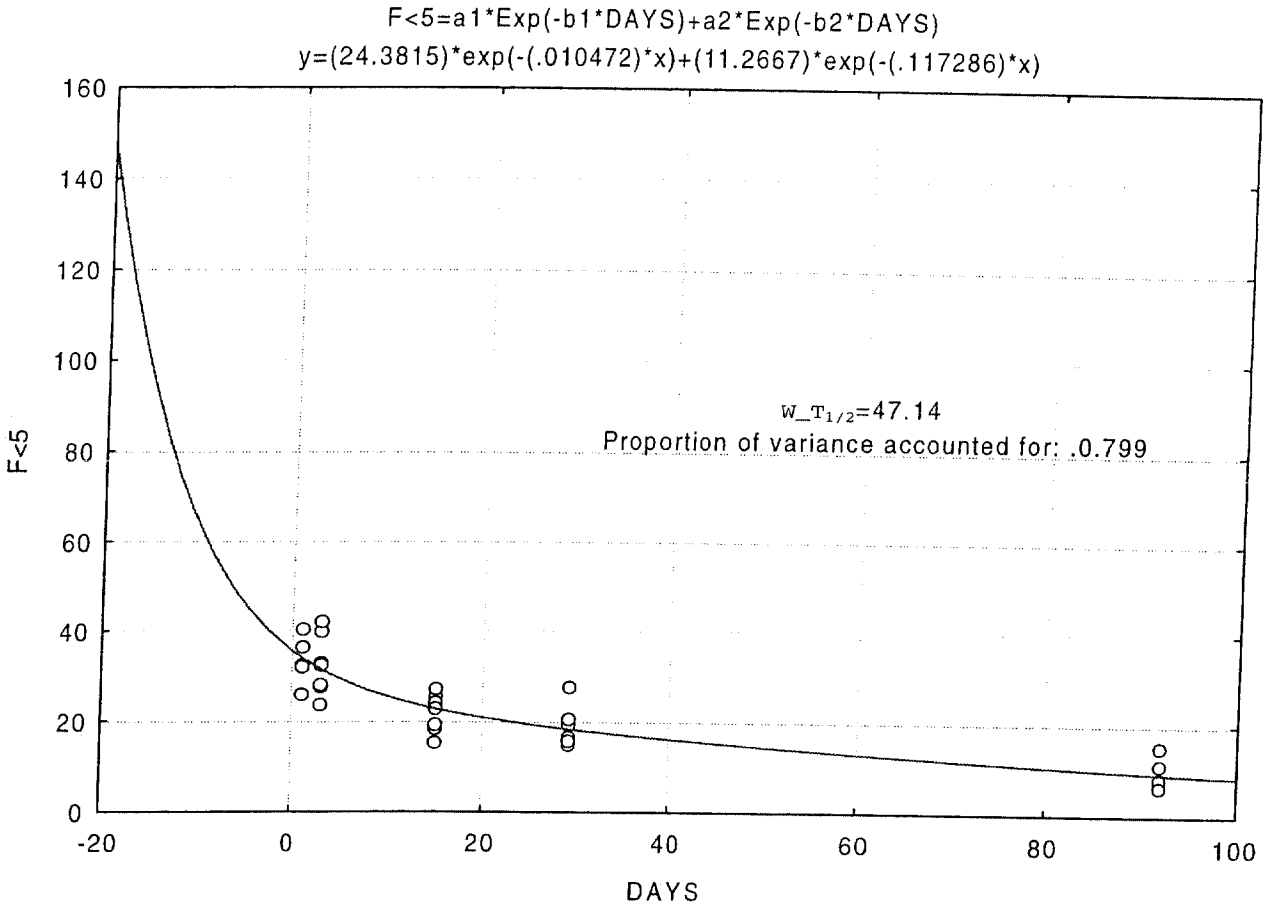
RCC STUDY NUMBER 801314
CARBON FIBRE

Number of WHO Fibres (Mio/lung)



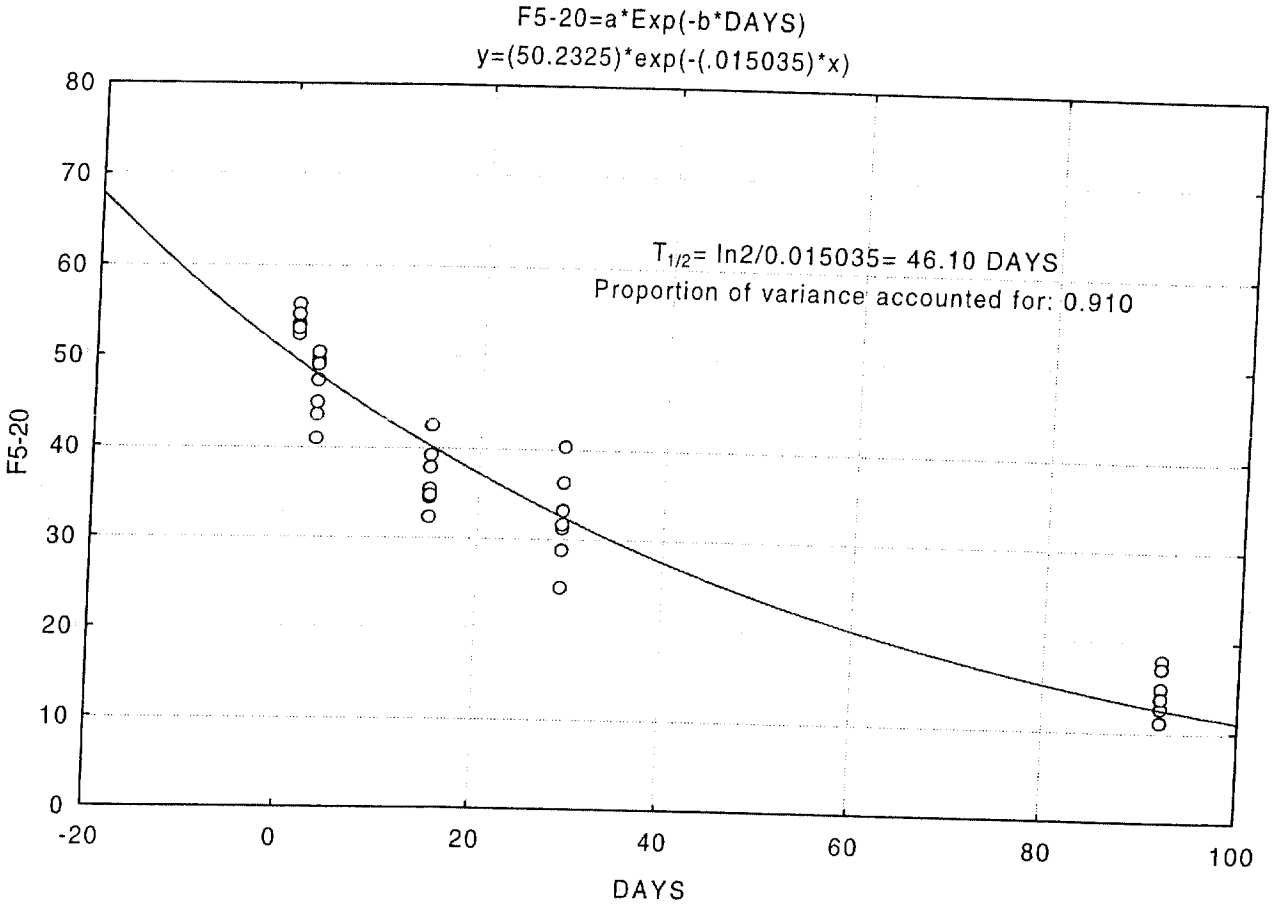
RCC STUDY NUMBER 801314
CARBON FIBRE

Number of Fibres < 5 µm (Mio/lung)



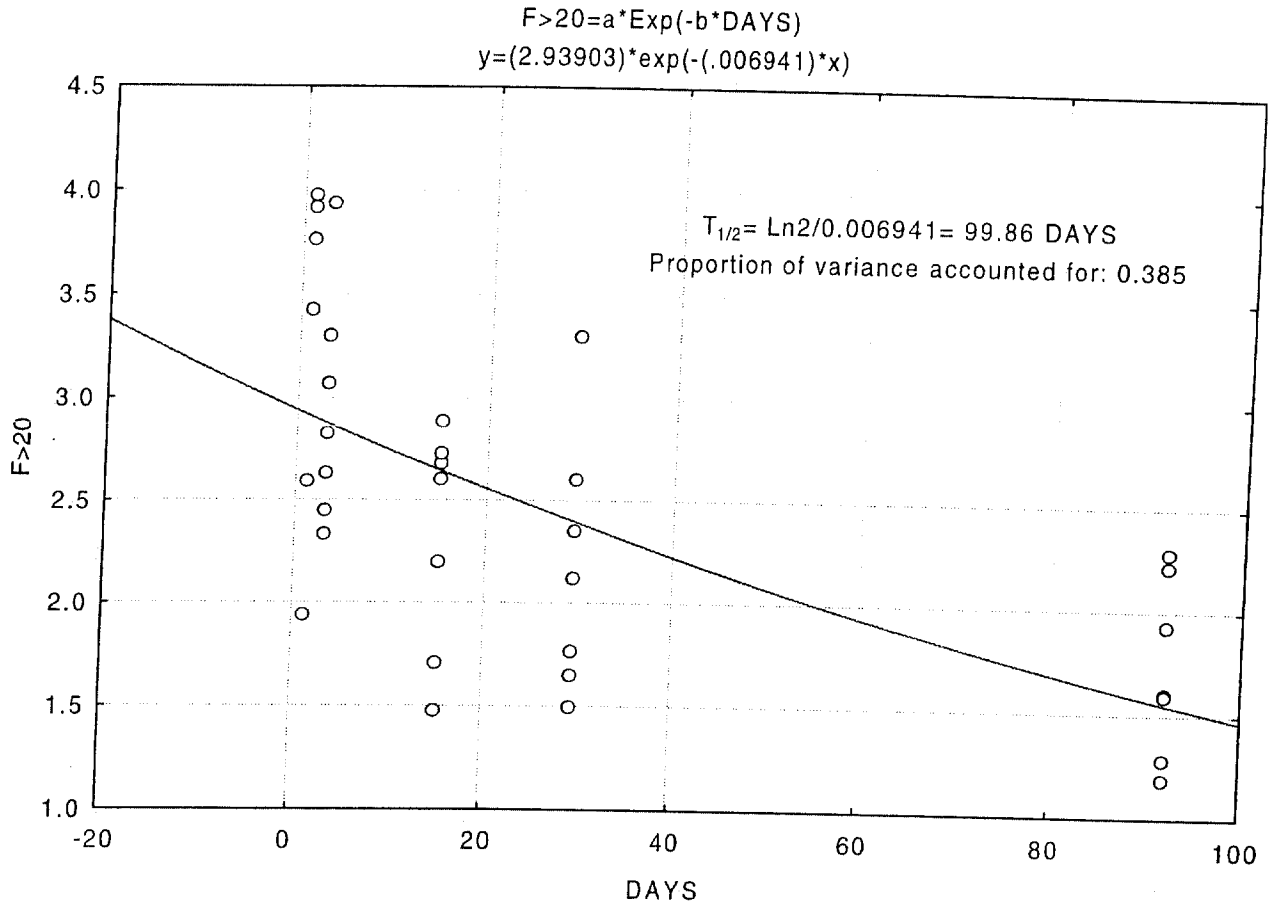
RCC STUDY NUMBER 801314
CARBON FIBRE

Number of Fibres between 5 and 20 µm (Mio/lung)



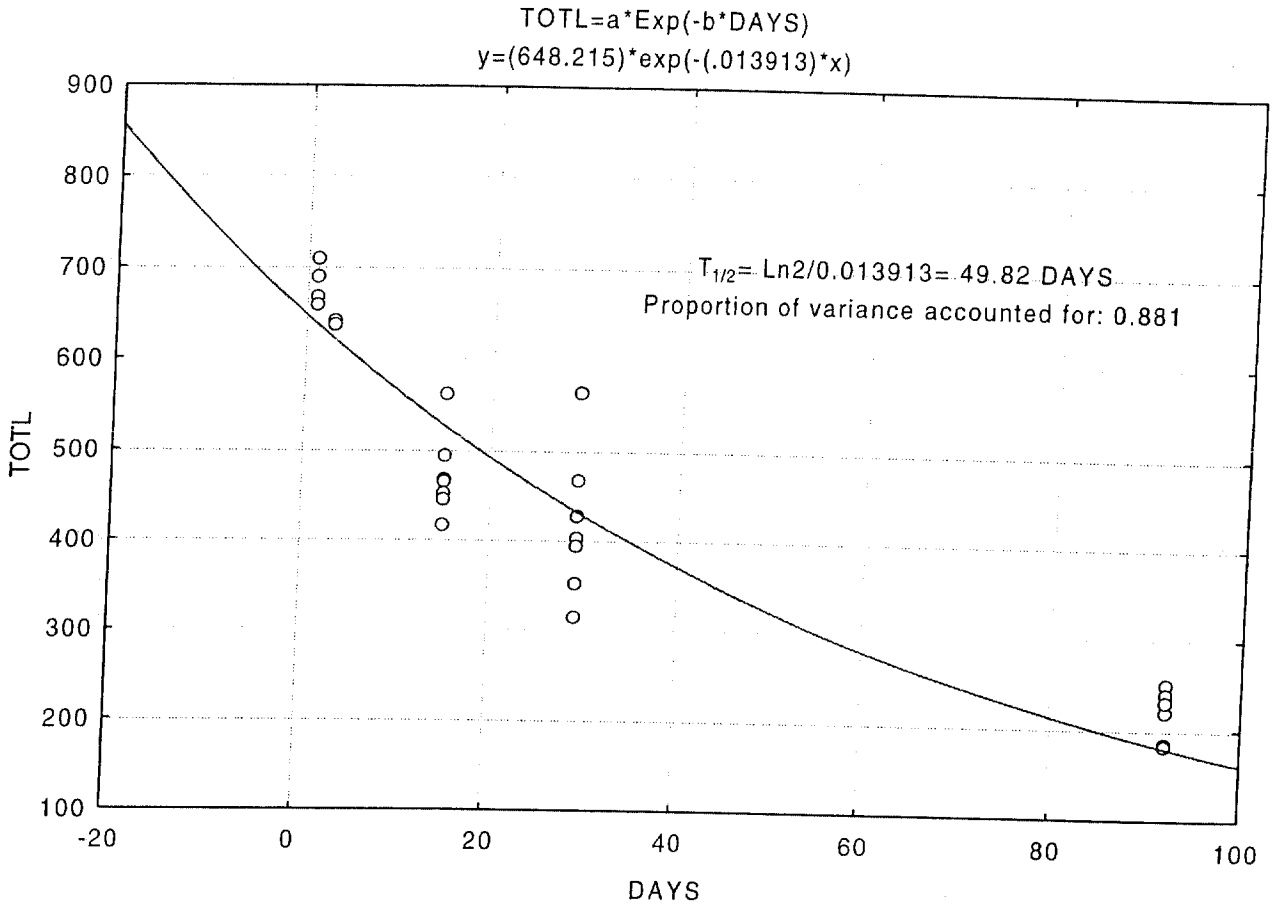
RCC STUDY NUMBER 801314
CARBON FIBRE

Number of Fibres longer than 20 µm (Mio/lung)



RCC STUDY NUMBER 801314
CARBON FIBRE

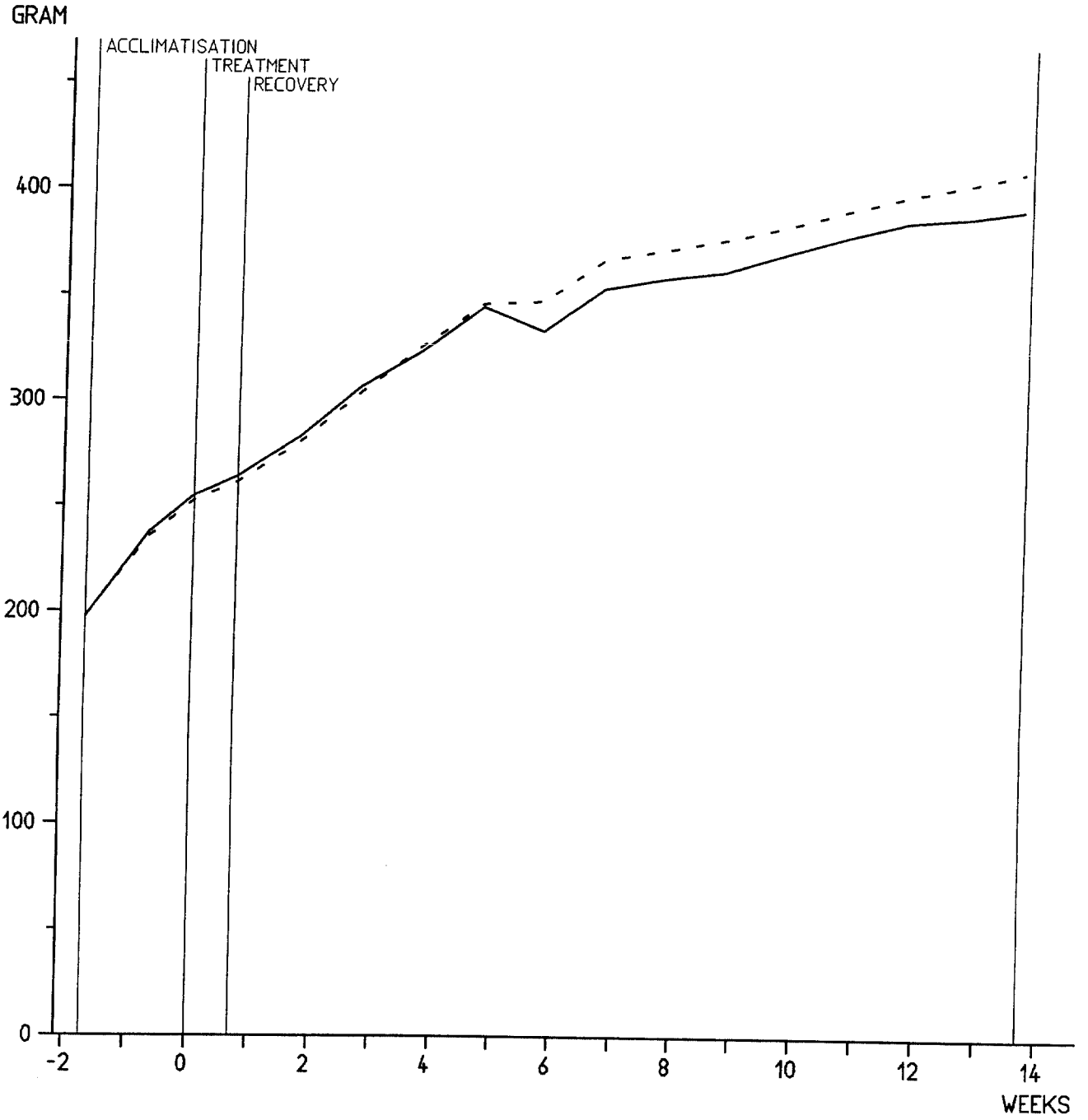
Total Length of Fibres / Lung (Metres)



RCC STUDY NUMBER 801314
CARBON FIBRE

BW-SPLT - 1
13-MAY-02

BODY WEIGHTS
MALES



GROUP 1 (AIR CONTROL)

GROUP 2 (CARBON FIBRE)

RCC STUDY NUMBER 801314
CARBON FIBRE

TABLES OF SUMMARY DATA

BODY WEIGHTS

ORGAN WEIGHTS

ORGAN WEIGHT RATIOS

MACROSCOPICAL FINDINGS

BODY WEIGHTS (GRAM) SUMMARY
MALES

ACCLIMATISATION		GROUP 1 AIR CONTROL	GROUP 2 CARBON FIBRE
DAY 1	MEAN	197.7	197.9
WEEK 1	ST.DEV.	7.3	6.9
	N	15	35
DAY 8	MEAN	237.2	235.4
WEEK 2	ST.DEV.	8.6	8.3
	N	15	35

* / ** : T-Test based on pooled variance significant at 5% (*) or 1% (**) level

BODY WEIGHTS (GRAM) SUMMARY
MALES

TREATMENT			GROUP 1 AIR CONTROL	GROUP 2 CARBON FIBRE
DAY 1		MEAN	254.5	252.4
WEEK 1		ST.DEV.	13.5	11.0
		N	15	35

* / ** : T-Test based on pooled variance significant at 5% (*) or 1% (**) level

BODY WEIGHTS (GRAM) SUMMARY
MALES

RECOVERY		GROUP 1 AIR CONTROL	GROUP 2 CARBON FIBRE
DAY 1	MEAN	263.8	261.1
WEEK 1	ST.DEV.	16.9	14.4
	N	15	35
DAY 8	MEAN	282.7	280.0
WEEK 2	ST.DEV.	18.9	17.2
	N	10	21
DAY 15	MEAN	306.7	303.7
WEEK 3	ST.DEV.	20.8	18.4
	N	10	21
DAY 22	MEAN	323.3	325.9
WEEK 4	ST.DEV.	23.7	16.5
	N	10	14
DAY 29	MEAN	344.5	346.1
WEEK 5	ST.DEV.	26.1	16.3
	N	10	14
DAY 36	MEAN	333.4	347.6
WEEK 6	ST.DEV.	11.2	15.8
	N	5	7
DAY 43	MEAN	353.7	367.1
WEEK 7	ST.DEV.	10.9	15.7
	N	5	7
DAY 50	MEAN	358.5	372.1
WEEK 8	ST.DEV.	11.6	16.8
	N	5	7
DAY 57	MEAN	362.1	377.2
WEEK 9	ST.DEV.	12.8	19.5
	N	5	7
DAY 64	MEAN	370.9	383.7
WEEK 10	ST.DEV.	12.7	20.5
	N	5	7
DAY 71	MEAN	379.3	391.9
WEEK 11	ST.DEV.	14.9	22.2
	N	5	7
DAY 78	MEAN	386.6	398.9
WEEK 12	ST.DEV.	13.0	23.0
	N	5	7
DAY 85	MEAN	389.2	405.0
WEEK 13	ST.DEV.	13.3	23.7
	N	5	7
DAY 91	MEAN	392.9	410.4
WEEK 13	ST.DEV.	13.3	23.9
	N	5	7

* / ** : T-Test based on pooled variance significant at 5% (*) or 1% (**) level

ORGAN WEIGHTS (GRAM) SUMMARY
AFTER 1-DAY OF RECOVERY
MALES

		GROUP 1 AIR CONTROL	GROUP 2 CARBON FIBRE
BODY W.	MEAN	261.288	258.363
	ST.DEV.	12.172	11.849
	N	5	7
TRACHEA	MEAN	0.125	0.109
	ST.DEV.	0.024	0.022
	N	5	7
LUNG(WHOLE)	MEAN	1.224	1.220
	ST.DEV.	0.087	0.040
	N	5	7
ALL LU.LOB	MEAN	1.103	1.112
	ST.DEV.	0.072	0.030
	N	5	7

ORGAN WEIGHTS (GRAM) SUMMARY
AFTER 3-DAYS OF RECOVERY
MALES

		GROUP 2 CARBON FIBRE
BODY W.	MEAN	262.266
	ST.DEV.	12.479
	N	7
TRACHEA	MEAN	0.114
	ST.DEV.	0.016
	N	7
LUNG(WHOLE)	MEAN	1.265
	ST.DEV.	0.072
	N	7
ALL LU.LOB	MEAN	1.150
	ST.DEV.	0.085
	N	7

ORGAN WEIGHTS (GRAM) SUMMARY
AFTER 15-DAYS OF RECOVERY
MALES

		GROUP 2 CARBON FIBRE
BODY W.	MEAN	293.880
	ST.DEV.	18.398
	N	7
TRACHEA	MEAN	0.098
	ST.DEV.	0.015
	N	7
LUNG(WHOLE)	MEAN	1.284
	ST.DEV.	0.089
	N	7
ALL LU.LOB	MEAN	1.185
	ST.DEV.	0.084
	N	7

ORGAN WEIGHTS (GRAM) SUMMARY
AFTER 29-DAYS OF RECOVERY
MALES

		GROUP 1 AIR CONTROL	GROUP 2 CARBON FIBRE
BODY W.	MEAN	344.676	341.766
	ST.DEV.	27.691	15.912
	N	5	7
TRACHEA	MEAN	0.118	0.143 *
	ST.DEV.	0.011	0.021
	N	5	7
LUNG(WHOLE)	MEAN	1.339	1.452 *
	ST.DEV.	0.102	0.056
	N	5	7
ALL LU.LOB	MEAN	1.217	1.307
	ST.DEV.	0.102	0.040
	N	5	7

ORGAN WEIGHTS (GRAM) SUMMARY
AFTER 92-DAYS OF RECOVERY
MALES

		GROUP 1 AIR CONTROL	GROUP 2 CARBON FIBRE
BODY W.	MEAN	385.302	401.319
	ST.DEV.	12.947	24.957
	N	5	7
TRACHEA	MEAN	0.131	0.131
	ST.DEV.	0.020	0.017
	N	5	7
LUNG(WHOLE)	MEAN	1.369	1.587 **
	ST.DEV.	0.105	0.085
	N	5	7
ALL LU.LOB	MEAN	1.239	1.449 **
	ST.DEV.	0.092	0.070
	N	5	7

ORGAN/BODY WEIGHT RATIOS SUMMARY
AFTER 1-DAY OF RECOVERY
MALES

		GROUP 1 AIR CONTROL	GROUP 2 CARBON FIBRE
BODY W. (GRAM)	MEAN	261.288	258.363
	ST.DEV.	12.172	11.849
	N	5	7
TRACHEA (%)	MEAN	0.048	0.042
	ST.DEV.	0.009	0.008
	N	5	7
LUNG(WHOLE) (%)	MEAN	0.469	0.473
	ST.DEV.	0.030	0.012
	N	5	7
ALL LU.LOB (%)	MEAN	0.422	0.431
	ST.DEV.	0.025	0.012
	N	5	7

ORGAN/BODY WEIGHT RATIOS SUMMARY
AFTER 3-DAYS OF RECOVERY
MALES

		GROUP 2 CARBON FIBRE
BODY W.	MEAN	262.266
(GRAM)	ST.DEV.	12.479
	N	7
TRACHEA	MEAN	0.044
(%)	ST.DEV.	0.007
	N	7
LUNG(WHOLE)	MEAN	0.483
(%)	ST.DEV.	0.036
	N	7
ALL LU.LOB	MEAN	0.439
(%)	ST.DEV.	0.038
	N	7

ORGAN/BODY WEIGHT RATIOS SUMMARY
AFTER 15-DAYS OF RECOVERY
MALES

		GROUP 2 CARBON FIBRE
BODY W.	MEAN	293.880
(GRAM)	ST.DEV.	18.398
	N	7
TRACHEA	MEAN	0.033
(%)	ST.DEV.	0.004
	N	7
LUNG(WHOLE)	MEAN	0.437
(%)	ST.DEV.	0.023
	N	7
ALL LU.LOB	MEAN	0.404
(%)	ST.DEV.	0.024
	N	7

ORGAN/BODY WEIGHT RATIOS SUMMARY
AFTER 29-DAYS OF RECOVERY
MALES

		GROUP 1 AIR CONTROL	GROUP 2 CARBON FIBRE
BODY W. (GRAM)	MEAN ST.DEV. N	344.676 27.691 5	341.766 15.912 7
TRACHEA (%)	MEAN ST.DEV. N	0.034 0.003 5	0.042 * 0.006 7
LUNG(WHOLE) (%)	MEAN ST.DEV. N	0.389 0.024 5	0.426 * 0.021 7
ALL LU.LOB (%)	MEAN ST.DEV. N	0.354 0.026 5	0.383 * 0.018 7

ORGAN/BODY WEIGHT RATIOS SUMMARY
AFTER 92-DAYS OF RECOVERY
MALES

		GROUP 1 AIR CONTROL	GROUP 2 CARBON FIBRE
BODY W.	MEAN	385.302	401.319
(GRAM)	ST.DEV.	12.947	24.957
	N	5	7
TRACHEA	MEAN	0.034	0.033
(%)	ST.DEV.	0.005	0.004
	N	5	7
LUNG(WHOLE)	MEAN	0.355	0.396 *
(%)	ST.DEV.	0.017	0.029
	N	5	7
ALL LU.LOB	MEAN	0.321	0.362 *
(%)	ST.DEV.	0.014	0.026
	N	5	7

MACROSCOPICAL FINDINGS SUMMARY
MALES
ALL NECROPSIES

	GROUP 1 AIR CONTROL		GROUP 2 CARBON FIBRE	
ANIMALS EXAMINED	15		35	
ANIMALS WITHOUT FINDINGS	15		28	
ANIMALS AFFECTED:				
LUNGS.....				
FOCUS/FOCI	0	0%	6	17%
NOT COLLAPSED	0	0%	3	9%

RCC STUDY NUMBER 801314
CARBON FIBRE

TABLES OF INDIVIDUAL DATA

GRAVIMETRIC CONCENTRATIONS

BODY WEIGHTS

ORGAN WEIGHTS

ORGAN WEIGHT RATIOS

MACROSCOPICAL FINDINGS

RCC STUDY NUMBER 801314
CARBON FIBRE

GRAVIMETRIC CONCENTRATIONS

Group 1 (Air Control)

Date	mg/m³
15.08.2001	0.055
16.08.2001	0.006
17.08.2001	0.000
18.08.2001	0.000
19.08.2001	0.000
Mean	0.012
SD	0.024

Group 2 (Carbon fibre)

Date	Stage	Aerosol Concentration (mg Carbon Fibre/m³)			
		Daily value	Stage 1	Stage 2	Stage 3
15.08.2001	1	15.7			
16.08.2001	1, 2, 3	14.2	14.3	14.2	14.1
17.08.2001	3	17.2			
18.08.2001	1	15.1			
19.08.2001	2	17.8			
Mean		16.0	Mean	14.2	
SD		1.48	SD	0.11	
CV (%)		9.2	CV	0.8	

16.08.2001 Simultaneous gravimetric samples on all three exposure levels

SD Standard deviation
CV (%) Coefficient of variation = SD/Mean x 100

BODY WEIGHTS (GRAM)
MALES

	ACCLIMATISATION		TREATMENT	RECOVERY			
DAYS	1	8	1	1	8	15	22
WEEKS	1	2	1	1	2	3	4
ANIMAL							
GROUP 1 (AIR CONTROL)							
1	199.9	234.6	248.8	254.5	---	---	---
2	207.2	245.8	266.6	279.4	---	---	---
3	190.7	235.3	259.0	270.2	---	---	---
4	204.8	240.9	266.3	275.3	---	---	---
5	195.3	232.6	249.8	258.9	---	---	---
6	186.6	235.3	253.6	269.0	288.7	316.9	332.4
7	198.5	236.2	252.5	258.6	277.3	291.2	301.0
8	200.0	249.0	280.4	301.5	320.8	347.6	371.3
9	206.3	244.6	259.8	268.4	285.7	316.5	344.0
10	198.1	244.4	261.0	263.0	289.9	314.8	332.1
11	195.0	235.7	246.8	251.5	275.5	298.3	317.4
12	188.2	223.8	235.6	247.3	267.9	288.3	302.4
13	185.6	217.9	225.4	230.1	253.8	279.2	294.8
14	205.3	247.1	266.0	278.7	300.0	323.3	331.6
15	203.8	235.5	246.2	249.9	267.1	290.6	305.9
GROUP 2 (CARBON FIBRE)							
16	195.6	234.5	252.8	272.5	---	---	---
17	200.6	239.7	254.8	262.2	---	---	---
18	183.8	220.9	235.2	243.0	---	---	---
19	189.5	225.3	249.5	262.4	---	---	---
20	206.6	239.6	257.3	266.7	---	---	---
21	208.8	250.3	271.8	285.4	---	---	---
22	205.8	224.0	256.4	266.5	---	---	---
23	195.6	224.8	236.2	241.0	---	---	---
24	202.1	236.7	256.3	264.7	---	---	---
25	205.6	244.0	264.8	276.7	---	---	---
26	204.2	240.0	255.4	261.6	---	---	---
27	205.2	242.7	259.9	271.5	---	---	---
28	198.4	239.8	248.6	258.0	---	---	---
29	204.2	240.4	255.1	263.5	---	---	---
30	186.3	232.7	245.6	252.7	274.6	300.4	---
31	190.1	230.7	246.6	248.2	263.7	284.6	---
32	202.1	246.1	269.1	287.6	308.0	340.1	---
33	197.1	228.0	243.0	250.4	260.4	279.2	---
34	202.2	234.5	247.2	254.7	268.8	296.9	---
35	192.6	230.7	248.1	251.8	258.9	280.5	---
36	194.2	229.3	234.9	233.0	257.7	281.1	---
37	190.4	229.9	248.0	259.7	280.2	305.0	324.9
38	198.8	254.2	275.1	294.3	320.1	341.7	359.4
39	199.5	236.2	264.7	276.7	299.1	323.1	345.9
40	189.3	228.6	254.8	263.4	285.5	303.6	317.1
41	195.8	235.4	244.6	254.2	276.2	302.1	318.4
42	192.5	239.7	253.1	265.6	287.1	306.7	322.9
43	187.4	222.2	237.9	242.2	273.7	302.3	321.4
44	205.9	243.0	255.6	263.9	283.5	308.6	325.8
45	202.8	235.9	248.0	252.9	277.9	307.4	328.5
46	204.0	250.2	280.2	290.2	310.2	336.5	353.4
47	199.7	232.7	251.3	257.7	285.0	302.3	320.9
48	198.3	231.3	241.7	248.1	267.8	292.2	312.4
49	186.4	223.5	240.2	245.4	269.8	289.1	300.0
50	205.7	239.9	248.9	252.0	271.4	295.4	311.1

BODY WEIGHTS (GRAM)
MALES

	RECOVERY								
DAYS	29	36	43	50	57	64	71	78	85
WEEKS	5	6	7	8	9	10	11	12	13
ANIMAL									
GROUP 1 (AIR CONTROL)									
1	---	---	---	---	---	---	---	---	---
2	---	---	---	---	---	---	---	---	---
3	---	---	---	---	---	---	---	---	---
4	---	---	---	---	---	---	---	---	---
5	---	---	---	---	---	---	---	---	---
6	356.6	---	---	---	---	---	---	---	---
7	317.0	---	---	---	---	---	---	---	---
8	401.5	---	---	---	---	---	---	---	---
9	363.5	---	---	---	---	---	---	---	---
10	354.1	---	---	---	---	---	---	---	---
11	334.0	341.2	358.5	369.4	373.9	380.7	389.4	396.2	397.3
12	323.1	329.0	347.5	350.1	348.5	355.3	360.0	370.1	372.9
13	316.9	318.7	342.7	346.8	355.3	365.2	373.1	380.9	384.5
14	347.8	347.6	370.4	372.5	377.6	386.9	398.2	402.8	407.4
15	330.3	330.4	349.6	353.9	355.3	366.5	375.6	383.1	383.9
GROUP 2 (CARBON FIBRE)									
16	---	---	---	---	---	---	---	---	---
17	---	---	---	---	---	---	---	---	---
18	---	---	---	---	---	---	---	---	---
19	---	---	---	---	---	---	---	---	---
20	---	---	---	---	---	---	---	---	---
21	---	---	---	---	---	---	---	---	---
22	---	---	---	---	---	---	---	---	---
23	---	---	---	---	---	---	---	---	---
24	---	---	---	---	---	---	---	---	---
25	---	---	---	---	---	---	---	---	---
26	---	---	---	---	---	---	---	---	---
27	---	---	---	---	---	---	---	---	---
28	---	---	---	---	---	---	---	---	---
29	---	---	---	---	---	---	---	---	---
30	---	---	---	---	---	---	---	---	---
31	---	---	---	---	---	---	---	---	---
32	---	---	---	---	---	---	---	---	---
33	---	---	---	---	---	---	---	---	---
34	---	---	---	---	---	---	---	---	---
35	---	---	---	---	---	---	---	---	---
36	---	---	---	---	---	---	---	---	---
37	351.4	---	---	---	---	---	---	---	---
38	378.4	---	---	---	---	---	---	---	---
39	367.3	---	---	---	---	---	---	---	---
40	336.7	---	---	---	---	---	---	---	---
41	338.6	---	---	---	---	---	---	---	---
42	343.7	---	---	---	---	---	---	---	---
43	348.4	---	---	---	---	---	---	---	---
44	344.3	354.0	371.9	373.6	379.8	387.7	393.1	402.4	410.2
45	351.9	354.3	377.1	385.8	394.5	401.7	414.4	422.3	428.1
46	367.5	375.9	395.4	401.4	410.0	417.1	425.7	432.8	440.8
47	337.1	346.0	362.8	369.3	372.0	378.2	388.4	391.6	400.5
48	328.6	332.8	350.0	354.3	355.1	359.0	364.2	375.1	378.5
49	321.4	329.1	353.8	355.7	358.7	363.9	369.6	369.9	377.1
50	329.6	341.0	358.8	364.7	370.2	378.2	387.8	398.4	399.5

BODY WEIGHTS (GRAM)
MALES

	RECOVERY
DAYS	91
WEEKS	13
ANIMAL	

GROUP 1 (AIR CONTROL)

1	---
2	---
3	---
4	---
5	---
6	---
7	---
8	---
9	---
10	---
11	401.0
12	381.0
13	382.9
14	412.2
15	387.5

GROUP 2 (CARBON FIBRE)

16	---
17	---
18	---
19	---
20	---
21	---
22	---
23	---
24	---
25	---
26	---
27	---
28	---
29	---
30	---
31	---
32	---
33	---
34	---
35	---
36	---
37	---
38	---
39	---
40	---
41	---
42	---
43	---
44	413.4
45	429.2
46	450.2
47	408.2
48	383.4
49	383.7
50	404.9

ORGAN WEIGHTS (GRAM)
AFTER 1-DAY OF RECOVERY
MALES

GROUP 1 (AIR CONTROL)

ANIMAL NUMBER	BODY W.	TRACHEA	LUNG(WHOLE	ALL LU.LOB
1	248.930	0.146	1.284	1.150
2	274.710	0.134	1.265	1.131
3	264.020	0.095	1.208	1.114
4	270.460	0.144	1.285	1.144
5	248.320	0.103	1.079	0.976

GROUP 2 (CARBON FIBRE)

ANIMAL NUMBER	BODY W.	TRACHEA	LUNG(WHOLE	ALL LU.LOB
16	261.650	0.157	1.266	1.107
17	258.560	0.101	1.227	1.128
18	236.070	0.094	1.144	1.054
19	254.400	0.111	1.233	1.128
20	260.930	0.106	1.207	1.098
21	275.820	0.105	1.253	1.148
22	261.110	0.090	1.211	1.120

ORGAN WEIGHTS (GRAM)
AFTER 3-DAYS OF RECOVERY
MALES

GROUP 2 (CARBON FIBRE)

ANIMAL NUMBER	BODY W.	TRACHEA	LUNG(WHOLE	ALL LU.LOB
23	239.280	0.129	1.228	1.102
24	262.110	0.101	1.387	1.284
25	275.520	0.137	1.168	1.029
26	260.920	0.128	1.247	1.118
27	276.320	0.102	1.307	1.205
28	257.180	0.098	1.299	1.200
29	264.530	0.106	1.218	1.110

ORGAN WEIGHTS (GRAM)
AFTER 15-DAYS OF RECOVERY
MALES

GROUP 2 (CARBON FIBRE)

ANIMAL NUMBER	BODY W.	TRACHEA	LUNG(WHOLE	ALL LU.LOB
30	288.440	0.099	1.298	1.203
31	298.630	0.114	1.203	1.090
32	330.270	0.123	1.427	1.309
33	270.010	0.094	1.225	1.132
34	287.540	0.082	1.315	1.229
35	294.980	0.095	1.345	1.242
36	287.290	0.081	1.173	1.088

ORGAN WEIGHTS (GRAM)
AFTER 29-DAYS OF RECOVERY
MALES

GROUP 1 (AIR CONTROL)

ANIMAL NUMBER	BODY W.	TRACHEA	LUNG(WHOLE	ALL LU.LOB
6	343.760	0.101	1.443	1.338
7	306.370	0.115	1.198	1.082
8	383.050	0.130	1.368	1.227
9	353.040	0.119	1.415	1.285
10	337.160	0.124	1.273	1.152

GROUP 2 (CARBON FIBRE)

ANIMAL NUMBER	BODY W.	TRACHEA	LUNG(WHOLE	ALL LU.LOB
37	339.630	0.133	1.435	1.283
38	367.680	0.151	1.512	1.356
39	358.680	0.161	1.480	1.324
40	326.860	0.131	1.422	1.289
41	325.480	0.168	1.530	1.359
42	333.520	0.106	1.376	1.269
43	340.510	0.148	1.412	1.267

ORGAN WEIGHTS (GRAM)
AFTER 92-DAYS OF RECOVERY
MALES

GROUP 1 (AIR CONTROL)

ANIMAL NUMBER	BODY W.	TRACHEA	LUNG(WHOLE	ALL LU.LOB
11	392.070	0.147	1.403	1.254
12	370.050	0.120	1.282	1.164
13	382.410	0.102	1.284	1.184
14	403.620	0.146	1.535	1.392
15	378.360	0.140	1.343	1.203

GROUP 2 (CARBON FIBRE)

ANIMAL NUMBER	BODY W.	TRACHEA	LUNG(WHOLE	ALL LU.LOB
44	405.780	0.110	1.596	1.486
45	422.670	0.128	1.634	1.472
46	442.150	0.141	1.544	1.401
47	400.320	0.160	1.731	1.564
48	375.140	0.138	1.617	1.476
49	373.550	0.115	1.493	1.378
50	389.620	0.122	1.491	1.369

ORGAN/BODY WEIGHT RATIOS (%)
AFTER 1-DAY OF RECOVERY
MALES

GROUP 1 (AIR CONTROL)

ANIMAL NUMBER	BODY W. (GRAM)	TRACHEA	LUNG(WHOLE ALL LU.LOB	
1	248.930	0.059	0.516	0.462
2	274.710	0.049	0.460	0.412
3	264.020	0.036	0.457	0.422
4	270.460	0.053	0.475	0.423
5	248.320	0.042	0.435	0.393

GROUP 2 (CARBON FIBRE)

ANIMAL NUMBER	BODY W. (GRAM)	TRACHEA	LUNG(WHOLE ALL LU.LOB	
16	261.650	0.060	0.484	0.423
17	258.560	0.039	0.475	0.436
18	236.070	0.040	0.485	0.446
19	254.400	0.044	0.485	0.443
20	260.930	0.041	0.463	0.421
21	275.820	0.038	0.454	0.416
22	261.110	0.035	0.464	0.429

ORGAN/BODY WEIGHT RATIOS (%)
AFTER 3-DAYS OF RECOVERY
MALES

GROUP 2 (CARBON FIBRE)

ANIMAL NUMBER	BODY W. (GRAM)	TRACHEA	LUNG(WHOLE	ALL LU.LOB
23	239.280	0.054	0.513	0.460
24	262.110	0.039	0.529	0.490
25	275.520	0.050	0.424	0.373
26	260.920	0.049	0.478	0.429
27	276.320	0.037	0.473	0.436
28	257.180	0.038	0.505	0.467
29	264.530	0.040	0.461	0.420

ORGAN/BODY WEIGHT RATIOS (%)
AFTER 15-DAYS OF RECOVERY
MALES

GROUP 2 (CARBON FIBRE)

ANIMAL NUMBER	BODY W. (GRAM)	TRACHEA	LUNG(WHOLE	ALL LU.LOB
30	288.440	0.034	0.450	0.417
31	298.630	0.038	0.403	0.365
32	330.270	0.037	0.432	0.396
33	270.010	0.035	0.454	0.419
34	287.540	0.029	0.457	0.427
35	294.980	0.032	0.456	0.421
36	287.290	0.028	0.408	0.379

ORGAN/BODY WEIGHT RATIOS (%)
AFTER 29-DAYS OF RECOVERY
MALES

GROUP 1 (AIR CONTROL)

ANIMAL NUMBER	BODY W. (GRAM)	TRACHEA	LUNG(WHOLE	ALL LU.LOB
6	343.760	0.029	0.420	0.389
7	306.370	0.038	0.391	0.353
8	383.050	0.034	0.357	0.320
9	353.040	0.034	0.401	0.364
10	337.160	0.037	0.378	0.342

GROUP 2 (CARBON FIBRE)

ANIMAL NUMBER	BODY W. (GRAM)	TRACHEA	LUNG(WHOLE	ALL LU.LOB
37	339.630	0.039	0.422	0.378
38	367.680	0.041	0.411	0.369
39	358.680	0.045	0.413	0.369
40	326.860	0.040	0.435	0.394
41	325.480	0.052	0.470	0.418
42	333.520	0.032	0.413	0.381
43	340.510	0.043	0.415	0.372

ORGAN/BODY WEIGHT RATIOS (%)
AFTER 92-DAYS OF RECOVERY
MALES

GROUP 1 (AIR CONTROL)

ANIMAL NUMBER	BODY W. (GRAM)	TRACHEA	LUNG(WHOLE	ALL LU.LOB
11	392.070	0.038	0.358	0.320
12	370.050	0.032	0.346	0.314
13	382.410	0.027	0.336	0.310
14	403.620	0.036	0.380	0.345
15	378.360	0.037	0.355	0.318

GROUP 2 (CARBON FIBRE)

ANIMAL NUMBER	BODY W. (GRAM)	TRACHEA	LUNG(WHOLE	ALL LU.LOB
44	405.780	0.027	0.393	0.366
45	422.670	0.030	0.387	0.348
46	442.150	0.032	0.349	0.317
47	400.320	0.040	0.432	0.391
48	375.140	0.037	0.431	0.393
49	373.550	0.031	0.400	0.369
50	389.620	0.031	0.383	0.351

MACROSCOPICAL FINDINGS
MALES
GROUP 1 (AIR CONTROL)

ANIMAL 1 (SCHEDULED NECROPSY, 20-AUG-2001)

NO FINDINGS NOTED

ANIMAL 2 (SCHEDULED NECROPSY, 20-AUG-2001)

NO FINDINGS NOTED

ANIMAL 3 (SCHEDULED NECROPSY, 20-AUG-2001)

NO FINDINGS NOTED

ANIMAL 4 (SCHEDULED NECROPSY, 20-AUG-2001)

NO FINDINGS NOTED

ANIMAL 5 (SCHEDULED NECROPSY, 20-AUG-2001)

NO FINDINGS NOTED

ANIMAL 6 (SCHEDULED NECROPSY, 17-SEP-2001)

NO FINDINGS NOTED

ANIMAL 7 (SCHEDULED NECROPSY, 17-SEP-2001)

NO FINDINGS NOTED

ANIMAL 8 (SCHEDULED NECROPSY, 17-SEP-2001)

NO FINDINGS NOTED

ANIMAL 9 (SCHEDULED NECROPSY, 17-SEP-2001)

NO FINDINGS NOTED

ANIMAL 10 (SCHEDULED NECROPSY, 17-SEP-2001)

NO FINDINGS NOTED

ANIMAL 11 (SCHEDULED NECROPSY, 19-NOV-2001)

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
MALES
GROUP 1 (AIR CONTROL)

ANIMAL 12 (SCHEDULED NECROPSY, 19-NOV-2001)

NO FINDINGS NOTED

ANIMAL 13 (SCHEDULED NECROPSY, 19-NOV-2001)

NO FINDINGS NOTED

ANIMAL 14 (SCHEDULED NECROPSY, 19-NOV-2001)

NO FINDINGS NOTED

ANIMAL 15 (SCHEDULED NECROPSY, 19-NOV-2001)

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
MALES
GROUP 2 (CARBON FIBRE)

ANIMAL 16 (SCHEDULED NECROPSY, 20-AUG-2001)

LUNGS..... FOCUS/FOCI, SEVERAL, D=1 MM, REDDISH.

ANIMAL 17 (SCHEDULED NECROPSY, 20-AUG-2001)

LUNGS..... NOT COLLAPSED.
FOCUS/FOCI, MANY, D=1 MM, REDDISH.

ANIMAL 18 (SCHEDULED NECROPSY, 20-AUG-2001)

NO FINDINGS NOTED

ANIMAL 19 (SCHEDULED NECROPSY, 20-AUG-2001)

LUNGS..... FOCUS/FOCI, SEVERAL, D=1 MM, GRAY WHITE.

ANIMAL 20 (SCHEDULED NECROPSY, 20-AUG-2001)

LUNGS..... FOCUS/FOCI, SEVERAL, D=1 MM, DARK RED.

ANIMAL 21 (SCHEDULED NECROPSY, 20-AUG-2001)

LUNGS..... FOCUS/FOCI, SEVERAL, D=1 MM, REDDISH.

ANIMAL 22 (SCHEDULED NECROPSY, 20-AUG-2001)

NO FINDINGS NOTED

ANIMAL 23 (SCHEDULED NECROPSY, 22-AUG-2001)

LUNGS..... NOT COLLAPSED.

ANIMAL 24 (SCHEDULED NECROPSY, 22-AUG-2001)

NO FINDINGS NOTED

ANIMAL 25 (SCHEDULED NECROPSY, 22-AUG-2001)

NO FINDINGS NOTED

ANIMAL 26 (SCHEDULED NECROPSY, 22-AUG-2001)

LUNGS..... NOT COLLAPSED.
FOCUS/FOCI, SEVERAL, D=1 MM, REDDISH.

MACROSCOPICAL FINDINGS
MALES
GROUP 2 (CARBON FIBRE)

ANIMAL 27 (SCHEDULED NECROPSY, 22-AUG-2001)

NO FINDINGS NOTED

ANIMAL 28 (SCHEDULED NECROPSY, 22-AUG-2001)

NO FINDINGS NOTED

ANIMAL 29 (SCHEDULED NECROPSY, 22-AUG-2001)

NO FINDINGS NOTED

ANIMAL 30 (SCHEDULED NECROPSY, 03-SEP-2001)

NO FINDINGS NOTED

ANIMAL 31 (SCHEDULED NECROPSY, 03-SEP-2001)

NO FINDINGS NOTED

ANIMAL 32 (SCHEDULED NECROPSY, 03-SEP-2001)

NO FINDINGS NOTED

ANIMAL 33 (SCHEDULED NECROPSY, 03-SEP-2001)

NO FINDINGS NOTED

ANIMAL 34 (SCHEDULED NECROPSY, 03-SEP-2001)

NO FINDINGS NOTED

ANIMAL 35 (SCHEDULED NECROPSY, 03-SEP-2001)

NO FINDINGS NOTED

ANIMAL 36 (SCHEDULED NECROPSY, 03-SEP-2001)

NO FINDINGS NOTED

ANIMAL 37 (SCHEDULED NECROPSY, 17-SEP-2001)

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
MALES
GROUP 2 (CARBON FIBRE)

ANIMAL 38 (SCHEDULED NECROPSY, 17-SEP-2001)

NO FINDINGS NOTED

ANIMAL 39 (SCHEDULED NECROPSY, 17-SEP-2001)

NO FINDINGS NOTED

ANIMAL 40 (SCHEDULED NECROPSY, 17-SEP-2001)

NO FINDINGS NOTED

ANIMAL 41 (SCHEDULED NECROPSY, 17-SEP-2001)

NO FINDINGS NOTED

ANIMAL 42 (SCHEDULED NECROPSY, 17-SEP-2001)

NO FINDINGS NOTED

ANIMAL 43 (SCHEDULED NECROPSY, 17-SEP-2001)

NO FINDINGS NOTED

ANIMAL 44 (SCHEDULED NECROPSY, 19-NOV-2001)

NO FINDINGS NOTED

ANIMAL 45 (SCHEDULED NECROPSY, 19-NOV-2001)

NO FINDINGS NOTED

ANIMAL 46 (SCHEDULED NECROPSY, 19-NOV-2001)

NO FINDINGS NOTED

ANIMAL 47 (SCHEDULED NECROPSY, 19-NOV-2001)

NO FINDINGS NOTED

ANIMAL 48 (SCHEDULED NECROPSY, 19-NOV-2001)

NO FINDINGS NOTED

MACROSCOPICAL FINDINGS
MALES
GROUP 2 (CARBON FIBRE)

ANIMAL 49 (SCHEDULED NECROPSY, 19-NOV-2001)

NO FINDINGS NOTED

ANIMAL 50 (SCHEDULED NECROPSY, 19-NOV-2001)

NO FINDINGS NOTED

RCC STUDY NUMBER 801314
CARBON FIBRE

ATTACHMENT 1

CHEMICAL ANALYSIS OF FOOD

ASSAY FOR CONTAMINANTS

WATER ANALYSIS

BACTERIOLOGICAL

CHEMICAL

CONTAMINANTS

RCC STUDY NUMBER 801314
CARBON FIBRE

ANALYTICAL TEST REPORT

RCC Project 813126
09.04.01

Prepared for	PROVIMI KLIBA AG 4303 Kaiseraugst
Attention of	Dr. Isler
Materials tested	Kliba 3433, Batch 71/01 vom 05.04.01
Test performed	AAS, GC, GC-MS, HPLC
Test results	See attached Table 1
Submitted	E. Dettwiler
Issued by	K. Biedermann

April 26, 2001/bon

The undersigned confirms that analysis of Kliba feed (number 3433, Batch 71/01, manufactured 05.04.01) was performed, and that this certificate represents accurately the analysis results of the feed delivered.

Date: April 26, 2001

PROVIMI KLIBA AG

RCC STUDY NUMBER 801314
CARBON FIBRE

ATTACHMENT

RCC Project 813126
09.04.01

Table 1 - Test Results

Kliba 3433, Batch 71/01
vom 05.04.01

PARAMETER	ASSAY LEVEL mg/kg	LIMIT* mg/kg
Aflatoxins (B1, B2, G1, G2), total	< 0.001	0.005
Estrogens (DES, Hexestrol, Dienestrol), total	< 0.001	0.001
Lindane	< 0.005	0.02
Heptachlor	< 0.005	0.02
Malathion	< 0.5	2.5
DDT, total	< 0.025	0.100
Dieldrin	< 0.005	0.02
Cadmium	0.04	0.160
Arsenic	0.15	1.0
Lead	0.33	1.5
Mercury	< 0.05	0.1
Selenium	0.18	0.6
Copper	13	----
PCBs	< 0.025	0.05
Nitrosamines (DMN, DEN, NPIP, NMORPH), total	< 0.01	0.010

< 0.001 = less than 0.001 milligram per kilogram
* = USP EPA, Federal Register, Vol. 44, No. 91, May 9, 1979

The original certificate of analysis has been archived by KLIBA of Kaiseraugst.

Date: April 26, 2001

PROVIMI KLIBA AG

RCC STUDY NUMBER 801314
CARBON FIBRE

ANALYTICAL TEST REPORT

RCC Project 826413
05.07.01

Prepared for	PROVIMI KLIBA AG 4303 Kaiseraugst
Attention of	Dr. Isler
Materials tested	Kliba 3433, Batch 73/01 vom 27.06.01
Test performed	AAS, GC, GC-MS, HPLC
Test results	See attached Table 1
Submitted	E. Dettwiler
Issued by	for K. Biedermann July 17, 2001/tre

The undersigned confirms that analysis of Kliba feed (number 3433, Batch 73/01, manufactured 27.06.01) was performed, and that this certificate represents accurately the analysis results of the feed delivered.

Date: July 17, 2001

PROVIMI KLIBA AG

RCC STUDY NUMBER 801314
CARBON FIBRE

ATTACHMENT

RCC Project 826413
05.07.01

Table 1 - Test Results

Kliba 3433, Batch 73/01
vom 27.06.01

PARAMETER	ASSAY LEVEL mg/kg	LIMIT* mg/kg
Aflatoxins (B1, B2, G1, G2), total	< 0.001	0.005
Estrogens (DES, Hexestrol, Dienestrol), total	< 0.001	0.001
Lindane	< 0.005	0.02
Heptachlor	< 0.005	0.02
Malathion	< 0.5	2.5
DDT, total	< 0.025	0.100
Dieldrin	< 0.005	0.02
Cadmium	0.04	0.160
Arsenic	< 0.15	1.0
Lead	0.38	1.5
Mercury	< 0.05	0.1
Selenium	0.40	0.6
Copper	14	----
PCBs	< 0.025	0.05
Nitrosamines (DMN, DEN, NPIP, NMORPH), total	< 0.01	0.010

< 0.001 = less than 0.001 milligram per kilogram
* = USP EPA, Federal Register, Vol. 44, No. 91, May 9, 1979

The original certificate of analysis has been archived by KLIBA of Kaiseraugst.

Date: July 17, 2001

PROVIMI KLIBA AG

RCC STUDY NUMBER 801314
CARBON FIBRE

BACTERIOLOGICAL ASSAY OF DRINKING WATER, FUELLINSDORF

Official Laboratory	Liestal, 19.07.2001
Basel-Landschaft	Ref.no. 200012250
Sampling point:	35.991.N Net water RCC Ltd, Füllinsdorf, Bldg. 2
Sampled on:	19.07.2001
Sample:	
Time of sampling	7.45-8.40
Water temperature (°C)	14.1

BACTERIOLOGICAL TEST:

Aerobic mesophilic bacteria / ml	0
E.coli / 100 ml	0
Enterococci / 100 ml	0

ASSESSMENT:

At the time of sampling, the tested bacteriological parameters met the requirements for drinking water according to article 275 of the "Eidg. Lebensmittelverordnung".

Official Laboratory
The Official Chemist

(signed Dr. N. Jäggi)

RCC STUDY NUMBER 801314
CARBON FIBRE

CHEMICAL WATER ANALYSIS, FUELLINSDORF

Official Laboratory Basel-Landschaft	Liestal, 19.07.2001 Ref.no. 200012251
Sampling point:	35.991.N Net water, RCC Ltd, Füllinsdorf, Bldg. 2
Sampled on:	19.07.2001
Time of sampling	7.45-8.40
Water temperature (°C)	14.1

CHEMICAL TEST:

Appearance			clear, colourless
Odor			not remarkable
Taste			not remarkable
UV-absorption at 254 nm/100 cm			1.56
Conductivity		µS/cm	598.0
Oxygen demand	($KMnO_4$ cons.)	mg/l	2.0
Total hardness		fr.H°	32.7
Alkaline hardness		fr.H°	24.2
Non carbonate hardness		fr.H°	8.5
Chloride	Cl^-	mg/l	14.6
Nitrate	NO_3^-	mg/l	18.2
Sulphate	SO_4^{--}	mg/l	67.2
Calcium	Ca^{++}	mg/l	117.7
Magnesium	Mg^{++}	mg/l	7.9
Nitrite	NO_2^-	mg/l	<0.005

ASSESSMENT:

At the time of sampling, the tested chemical parameters met the requirements for drinking water according to article 275 of the "Eidg. Lebensmittelverordnung".

Official Laboratory
The Official Chemist

(signed Dr. N. Jäggi)

RCC STUDY NUMBER 801314
CARBON FIBRE

CONTAMINANT ASSAY OF DRINKING WATER, RCC Ltd, FÜLLINSDORF

RCC Project: 904994

Date of Sampling: 19.07.2001

Sample: H_2O, RCC Ltd, Füllinsdorf, Bldg. 2

PARAMETER	ASSAY LEVEL µg/l	LIMIT * µg/l
Lindane	< 0.05	0.1
Heptachlor	< 0.05	0.1
Malathion	< 0.05	0.1
DDT, total	< 0.05	0.1
Dieldrin	< 0.05	0.1
Cadmium	< 0.5	5
Arsenic	< 3	50
Lead	< 3	50
Mercury	< 1	1
Selenium	< 3	10
Copper	< 4	1500
PCBs (28, 52, 101, 138, 153, 180)	< 0.05	0.1
Nitrosamines, total (DMN, DEN, NPIP, NMORPH)	< 0.05	-----

< 0.05 = less than 0.05 microgram per liter

* Schweizer Lebensmittelbuch

August 03, 2001

signed K. Biedermann

RCC STUDY NUMBER 801314
CARBON FIBRE

ATTACHMENT 2

GSA REPORT

GSA Gesellschaft für Schadstoffmessung und Auftragsanalytik GmbH
Gut Vellbrüggen D-41469 Neuss-Norf Tel.02137/918001 Fax.02137/4935

REPORT

GSA-Project: RCC 801314

CHARACTERISATION OF
CARBON FIBRES
FROM STUDY:
3-MONTHS BIOPERSISTENCE OF FIBRES IN RATS
FOLLOWING INHALATION

Part of the RCC Study Number 801314:
CARBON FIBRE
3-Month Biopersistence Inhalation Study in the Rat.

Version: **Final Report**
Date: 11-Sept-2002

Confidentiality Statement

Contents

Summary

At RCC/Fuellinsdorf fibres were administered to rats by inhalation. As test item one type of Carbon fibres was used. The objective for GSA was to characterise the test item at different stages of the project by determining the size distribution from fibres and the fibre number concentrations. Counting and sizing were done by means of a scanning electron microscope (SEM) with energy dispersive x-ray analysis (EDS).

The investigations performed are summarised in Table 0.1 to 0.2.

Table 0.1: Test Matrix for aerosol

	Group	
	Air Control	Carbon fibre
Exposure day	Number of samples for BVA and F/cc	
1	1	1
2	1	1
3	1	1
4	1	1
5	1	1

Table 0.2: Test Matrix for Lung Burden

	Group	
	Air Control	Carbon Fibre
post-exposure day	Number of samples for BVA and F/Lung	
1	5	7
3	-	7
15	-	7
29	5	7
92	5	7

BVA = Bivariate analysis of fibre size distribution
F/cc = Determination of fibre concentration (fibre number per cubic centimetre of air)
F/Lung = Determination of fibre number per lung

The summaries of the results are given in the table 0.3 to 0.4.

Table 0.3 Air Control

Origin of sample	Sacrifice time point	Fibres L > 20 µm	WHO[1]-fibres	Total fibres		
		Number per cm^3			Geom. mean diameter [µm]	Geom. mean length [µm]
Aerosols	N.A.	0,000	0,000	0,014	1,04	3,67
Lungs		Number per lung in millions				
	1 day	0,000	0,000	0,041	1,11	5,52
	29 days	0,000	0,000	0,036	1,31	7,15
	92 days	0,000	0,000	0,082	0,79	4,22

N.A. = not applicable

[1]) WHO definition means length (L) > 5 µm, diameter (D) < 3 µm and L:D ≥ 3.

Table 0.4: Carbon Fibre

Origin of sample	Sacrifice time point	Fibres L > 20 µm	WHO[1]-fibres	Total fibres		
		Number per cm^3			Geom. mean diameter [µm]	Geom. Mean length [µm]
Aerosols	N.A.	92,050	1046,079	1611,188	0,92	6,74
lungs		Number per lung in millions				
	1 day	3,049	51,676	83,858	0,87	6,24
	3 days	2,940	48,074	82,122	0,86	6,02
	15 days	2,332	37,825	61,764	0,89	6,26
	29 days	2,191	33,259	53,500	0,88	6,39
	92 days	1,734	15,705	25,378	0,89	6,62

N.A. = not applicable

[1]) WHO definition means length (L) > 5 µm, diameter (D) < 3 µm and L:D ≥ 3.

Statement of Compliance

GSA is not a GLP certified Laboratory. But GSA is

- nominated as an institute for measurements at workplace by the federal ministry of labour for sampling and counting fibres
- Co-worker in the German System for Accreditation (Deutsches Akkreditierungsystem Prüfwesen, DAP).

GSA states that, wherever possible, the investigations were conducted in compliance with:

- Grundsätze der Guten Laborpraxis [Principles of Good Laboratory Practice] (GLP), Bonn, March 22, 1990
- OECD Principles of Good Laboratory Practice, as revised in 1997 [C(97)186/(Final)].

Principal Investigator:

Date: 11.9.02 .....

(U. Teichert)

For the Sponsor:

Date: .....

.....

(Mr. Yasunobu Kikui)

Preface

General Information

Title:	CHARACTERISATION OF CARBON FIBRES FROM STUDY: 3-MONTHS BIOPERSISTENCE OF FIBRES IN RATS FOLLOWING INHALATION
Sponsor:	OSAKA GAS AMERICA INC. Pasadena California 91101, USA
Sponsor Representative:	Yasunobu Kikui (same address as above)
Monitoring Scientist and scientific advisor to the Sponsor:	Prof. Dr. Oberdörster 121 Southern Parkway Rochester, New York 14618, USA
Test Facility:	GSA Gesellschaft für Schadstoffmessung und Auftragsanalytik GmbH Gut Vellbrüggen D-41469 Neuss-Norf
GSA Project	RCC 801314
Test item:	Carbon fibre
Biological samples supplied by:	RCC Ltd, Toxicology Division Woelferstrasse 4 CH-4414 Fuellinsdorf RCC Ltd, Environmental Chemistry and Pharmanalytics Division Zelgliweg 1 CH-4452 Itingen

Schedule

Start of investigations:	August 2001
End of investigations:	May 2002

Archiving

GSA Neuss will archive the following data for at least ten years:
All raw data, copy of study plan, report, duplicate of report.
The remaining substance of the stock samples were stored in the archive of GSA under room conditions.
The measuring filters sampled with the suspension for SEM-analysis were stored in the archive of GSA under room conditions.
The remaining parts of the in GSA produced suspensions of the digested lung samples and the aerosol filters were stored in the archive of GSA in a refrigerator at a temperature of 5 to 8 °C until the expiration date.
Thereafter, no raw data, copy of study plan, report, duplicate of report and test item reference sample will be withdrawn without the sponsor's further consent.

At the end of 10 years (deviating from German GLP), the Sponsor will be contacted for the disposal of the archived material.
However on his request part or all archived material and documents can be sent to the Sponsor prior to the end of the 10-year period. Transportation and further archiving will occur under the responsibility of the Sponsor.

Quality Assurance Unit

GSA Gesellschaft für Schadstoffmessung und Auftragsanalytik GmbH D-41469 Neuss

Statement

GSA Project Number	RCC 801314
Test item	Carbon fibre
Principal Investigator	Dipl.-Ing. U. Teichert
Title	CHARACTERISATION OF CARBON FIBRES FROM STUDY: 3-MONTHS BIOPERSISTENCE OF FIBRES IN RATS FOLLOWING INHALATION

The validity of the present statement is limited to the work performed at GSA/Neuss.

Dates of QAU Inspections / Audits	Dates of Reports to the Principal Investigator and the management
06.08.2001	06.08.2001
22.08.2001	24.08.2001
24.08.2001	24.08.2001
28.08.2001	28.08.2001
04.09.2001	05.09.2001
05.04.2002	05.04.2002
09.04.2002	09.04.2002
22.04.2002	24.04.2002
23.04.2002	24.04.2002
24.04.2002	24.04.2002
02.05.2002	02.05.2002
13.05.2002	13.05.2002
11.09.2002	11.09.2002

Manager, Quality Assurance Unit:

Date: 11.9.2002

.....

(P. Kunzendorf)

Guidelines

GLP Guidelines

These investigations were carried out with the limitations given under Statement of Compliance in accordance with:

- Grundsätze der Guten Laborpraxis [Principles of Good Laboratory Practice] (GLP), Bonn, March, 22, 1990.
- OECD Principles of Good Laboratory Practice, as revised in 1997 [C(97)186/(Final)].

Test Guidelines

- EU document ECB/TM/26 rev.7, 1998
- WHO, World Health Organisation, Reference Methods For Measuring Airborne Man Made Mineral Fibres (MMMF), prepared by the WHO Regional Office for Europe, Copenhagen (1985)

Project Staff Signature

Principal Investigator:

Date: 11.9.02 .....

(U. Teichert)

Introduction

GSA received from RCC different kinds of samples used in the animal experiment in order to determine the fibre number concentration and/or the fibre size distribution.

Laboratory Methods

Documentation and Data Storage

For each batch of samples received from RCC a visual inspection of the parcel and of each sample container was carried out. Date and time of arrival, visual impression of the parcel's outside and inside and the identification of attached letters were recorded.
The by RCC attached chain of custody form was signed and dated. Relevant observations were noted. A copy was sent to RCC by fax in order to confirm the arrival of the samples.
For internal handling of the sample at GSA, each sample, identified with RCC-Codes, was given additionally a unique GSA-No . A block of numbers starting with 186500 was reserved for this project. For each sample an individual data sheet was prepared immediately after arriving at GSA. This sheet contains RCC and GSA sample identification, ordered analysis, dates and signatures of the persons which fulfilled the data sheet. Later on dates of preparation and analytic and signatures of the persons involved were added.

Sample Handling and Storage

Immediately after the samples were registered and the necessary analytical steps were defined, the samples were transferred into the lab together with the individual sample sheet. Suspensions were placed in a refrigerator and stored there at a temperature of less than 8 °C.
Each step of preparation and analysis as well as abnormalities, if any, in addition to the signatures of the persons involved was recorded in the individual data sheet.

General Description of Samples and Analytical Investigations

Samples

GSA received from RCC the following types of samples of the animal experiment:

a) Filters with the administered aerosols dry in a flask.

b) Filters with digested lung samples dry in flask.

Analytical Investigations

- determination of fibre number concentration in administered aerosols
- determination of fibre dimensions in administered aerosols
- determination of fibre number concentration in lungs
- determination of fibre dimensions in lungs

Analytical Methods

Sample Preparations

Aerosol Samples and digested lung samples

80 cm^3 of methanol were added to the flask containing the filters with the aerosol filters or the digested lung samples. The suspension was treated by sonication in an ultrasonic bath (35 kHz, 120 W) for 12 minutes. By doing this, the dust on the filter moved from the filter into the liquid. The filter was taken out of the flask and was washed with 10 cm^3 of methanol. This methanol was transferred back to the flask and resuspended by sonication in an ultrasonic bath (35 kHz, 120 W) for 3 minutes. An appropriate aliquot was sampled with a Gilson pipette immediately after and was diluted in 15 cm^3 of bidistilled and filtered water.

Filtration

Using a Millipore® funnel, the aqueous dispersion was filtered under moderate vacuum on a gold coated polycarbonate filter (Nuclepore®, diameter 25 mm, pore size 0.2 µm).The support filter and the measuring filter were tightly fastened into the filtration device. The filter surface was

moistened with a few drops of isopropanol. Then, as soon as the ultrasonic treatment was finished, an aliquot of the suspension was sucked into a pipette and transferred onto the filter with the addition of 1 cm^3 ethyl-alcohol. As optimum volume of the aliquot 0,5 cm^3 was judged from preliminary experiments. The liquid was sucked through the filter by means of a vacuum pump, and the solids were deposited on the surface of the filter. The ethyl-alcohol acts as an emulsifier to reduce surface tension and ensures an even distribution on the filter.

Shortly before the end of the filtration process, the inner surfaces of the filtration device above the measuring filter, which have come into contact with the suspension, were rinsed off with filtered bidistilled water from a wash bottle. The rinsing liquid was sucked through the filter together with the rest of the liquid. Finally the filter was dried in a drying chamber at 50°C without air circulation.

A report was made for each filtration. In this report all necessary data for subsequently calculating the concentration and for checking the work was recorded such as:

- designation of sample
- effective filter surface area
- volume of filtered suspension
- any special observations
- date of filtration
- name of operator

The loading of the filters with fibres and particles was checked. Only filters, whose loading allowed reliable examinations, were used. Filters too heavily loaded were rejected and a new filtration with a smaller aliquot took place. In case the filter was too poorly loaded, a new filtration with a greater aliquot was carried out.

Preparation of the filters

The gold-coated filter was fixed with conductive carbon cement, the dust loaded side up, on a filter holder of aluminium, which was pre coated with conductive carbon cement.

Determination of Fibre Dimensions

Unless otherwise specified, the basis for the evaluation using the scanning electron microscope was WHO-Reference Methods for measuring airborne man made mineral fibres (MMMF), prepared by the WHO Regional Office for Europe, Copenhagen (1985).

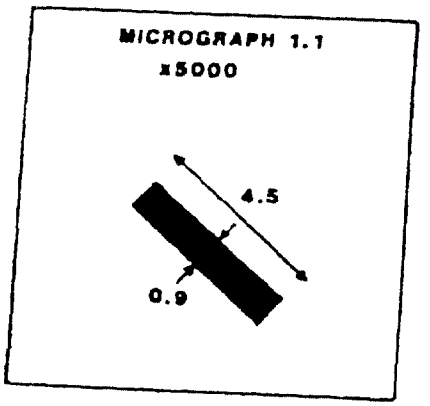
Normally the evaluation was carried out in the SEM at a magnification of 2000x. All objects which could be seen at this magnification were taken into consideration. At a magnification of 2000x the visibility is limited to fibres with a diameter of at least 0.1 µm.

The acceleration voltage was set to 25 kV.

The fields of view to be evaluated were chosen in such a way that the entire surface of the prepared filter was examined and that no overlapping of the fields of view being counted could occur.

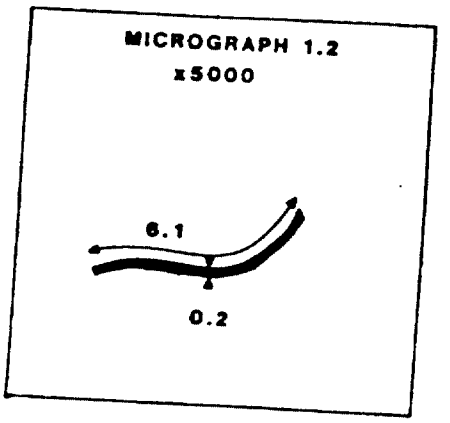
Each field of view was examined for fibres which met the criteria of length and diameter described below.

- All fibres, defined as all objects with an aspect ratio of at least 3:1, visible at a magnification of 2000x had to be taken in consideration. All fibres which were seen at this magnification were sized. No lower or upper limit was imposed on neither length nor diameter. All other objects with the same chemical composition as the fibres were defined as particles. They were measured in the same way as fibres as described below.
- Fibres with both ends in the field of view had the "weight" of one fibre (=2 fibre ends), fibres with only one end within the field had the "weight" of a half fibre (=1 fibre end). Fibres without any fibre end in the field of view had the "weight" of no fibre and were not counted nor measured.
- The number of the field of view in which the fibre was detected was recorded with the fibre "weight" and the individual bivariate length and diameter for each object measured



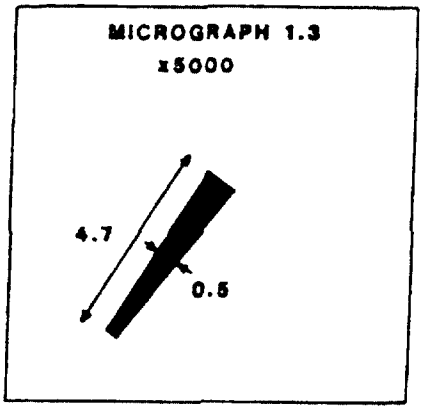
Field 1; weight 1; length 4,5 µm; diameter 0,9 µm

– Where fibres were curved, the length was measured as if the fibre were straightened out.



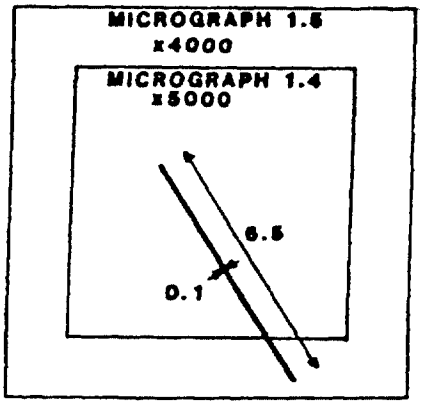
Field 2; weight 1; length 6,1 µm; diameter 0,2 µm

– Where a fibre did not have parallel sides, the diameter was measured at an "average" point.



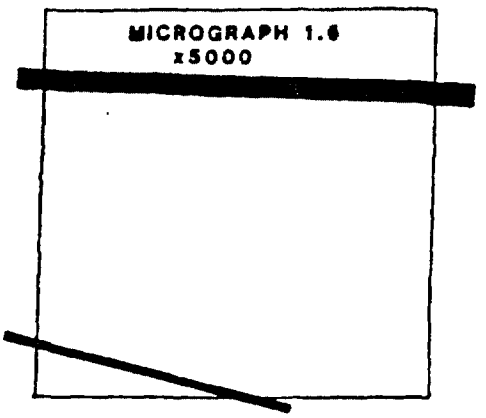
Field 3; weight 1; length 4,7 µm; diameter 0,5 µm

– If there was only one end within the field the measurements were made with a lower magnification. These measurements had the "weight" of a half fibre (one fibre end).

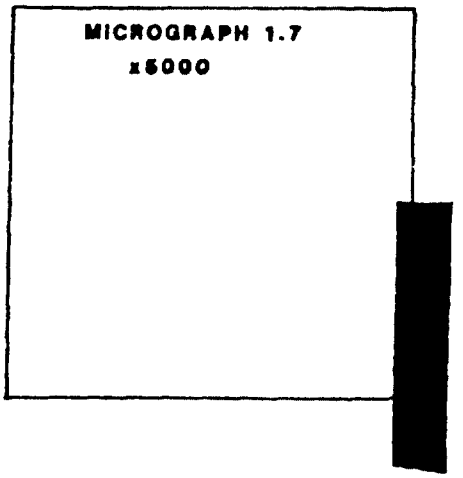


Field 4; weight 1/2; length 6,5 µm; diameter 0,1 µm

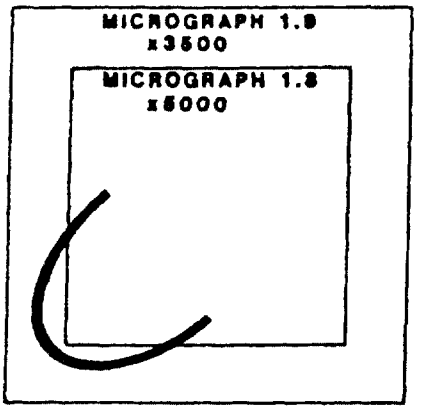
– If there were fibres with neither of there ends within the field, these fibres were not measured.



– A fibre end was defined as the midpoint of the fibre breadth at the extremity of the fibre. In the picture, the fibre end is not within the field and should not be measured.

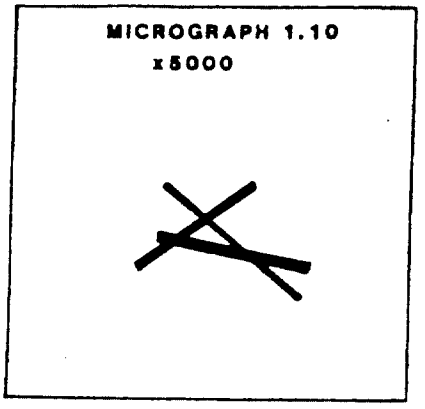


- If fibres crossed out of the field of view but have both ends within were measured and were given the weight of 1 fibre.



Field 7; weight 1; length 11 μm; diameter 0,2 μm

- Where fibres were grouped together, each constituent fibre was distinguished on the basis of continuity and measured separately.

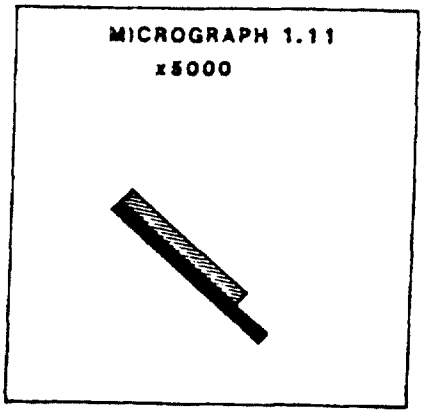


Field 8; weight 1; length 4,5 μm; diameter 0,1 μm

Field 8; weight 1; length 3,6 μm; diameter 0,2 μm

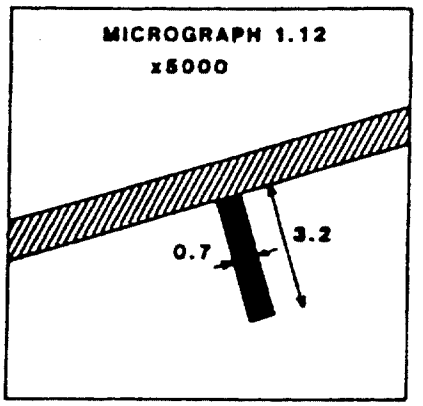
Field 8; weight 1; length 3,8 μm; diameter 0,3 μm

- In many cases, two or more fibres were seen to lie parallel to each other. Each fibre was measured.

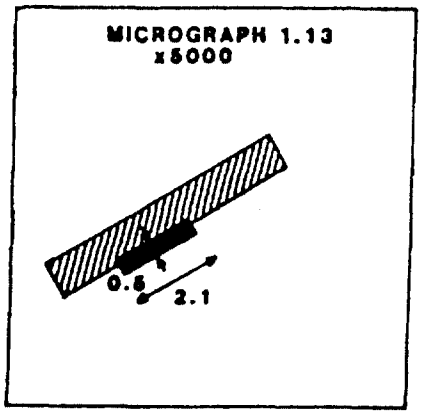


Field 9; weight 1; length 4,0 µm; diameter 0,3 µm
Field 9; weight 1; length 5,1 µm; diameter 0,3 µm

- Where two fibres were in contact, one may partially obscure the other. In these circumstances, the observed image was measured.



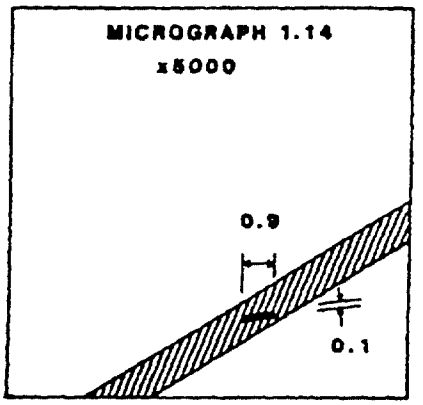
Field 10; weight 1; length 3,2 µm; diameter 0,7 µm



Field 11; weight 1; length 6,5 µm; diameter 0,9 µm

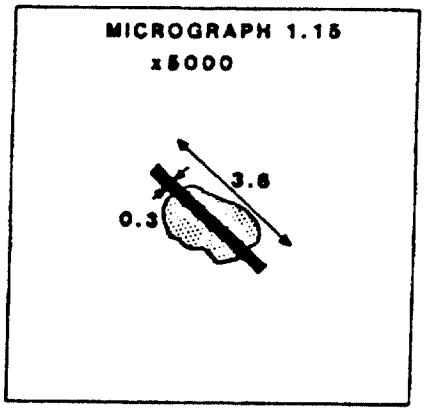
Field 11; weight 1; length 2,1 µm; diameter 0,5 µm

– In some circumstances, fibres were observed to lie directly on top of other fibres. Each fibre was measured as a distinct fibre.

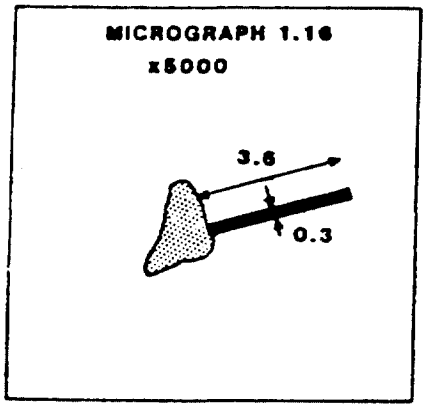


Field 12; weight 1; length 0,9 µm; diameter 0,1 µm

- Particles in contact with fibres were ignored and the visible part of the fibre was sized according to the rules given in the preceding sections. Fibres only partially obscured were measured on the basis of continuity.



Field 13; weight 1; length 3,6 µm; diameter 0,3 µm



Field 14; weight 1; length 3,6 µm; diameter 0,3 µm

The examination of the samples was stopped:

- Fibrous particles:

  So many fields of view were examined until a total of 400 WHO (length > 5 µm, diameter < 3 µm) fibres (800 fibre ends) or a total of 1000 objects was recorded or a maximum of 1 mm^2 of the filter surface was examined in case 400 countable WHO fibres was not reached.

- Non-fibrous particles:

  So many fields of view were examined until the a.m. stopping criteria for fibres were reached or a total of 100 particles were recorded.

- For samples of the control group only an area of 0,5 mm^2 of the filter was evaluated.

Sizing Report

A sizing report was set up for each sample evaluation. In this report all necessary data were recorded, such as:

- designation of sample
- effective filter surface area
- volume of filtered medium
- area of a field of view
- length and diameter of each fibre
- "weight" of each fibre
- number of fields of view in which the object was detected
- number of fields totally evaluated
- remarks in case of special observations
- date of evaluation
- name of counter

Calculation of the Fibre Number Concentration

The fibre number concentration in the suspensions was calculated from the data gained by sizing as follows:

$$C = n_{fibres} \times \frac{A_{filter}}{A_{ev}} \times \frac{1}{V_{filtered}} \qquad \text{equation (1)}$$

or

$$C = n_{fibres} \times \frac{A_{filter}}{n_{field} \times A_{screen}} \times \frac{1}{V_{filtered}} \qquad \text{equation (2)}$$

C = concentration [fibres/cm³ filtered suspension]

n_{fibres} = number of fibres sized

A_{filter} = effective filter surface area [mm²]

n_{field} = number of fields of view evaluated

A_{ev} = filter area evaluated [mm²]

A_{screen} = area of field of view [mm²]

$V_{filtered}$ = volume of filtered medium [cm³]

The number of fibres per filter was calculated as follows:

$$C_{filter} = C \times V_{meth}$$ equation (3)

C_{filter} = number of fibres on the whole original filter

V_{meth} = volume of total suspension (80+10=90 cm³)

The number of fibres per cm³ of air was calculated as follows:

$$C_{air} = C_{filter} \div V_{air}$$ equation (4)

C_{air} = fibres per cm³ of air

V_{air} = volume of air sampled

The number of fibres per lung was calculated as follows:

$$C_{lung} = C \times V_{susp}$$ equation (5)

C_{lung} = number of fibres per lung

V_{susp} = volume of total suspension (90 cm³)

$$C_{drylung} = C_{lung} / m_{drylung}$$ equation (6)

$m_{drylung}$ = weight of the dry lung (mg)

$C_{drylung}$ = number of fibres per mg dry lung

C_{lung} = number of fibres per lung

The weight of the dry lungs was determined at RCC.

Calculation of the mean fibre dimensions

The arithmetic mean diameter was calculated as follows:

$$D_{arith.\ mean..} = \Sigma D / \Sigma n \qquad \text{equation (7)}$$

ΣD =sum of all recorded diameters for each time point and fibre type
Σn =sum of all recorded fibres for each time point and fibre type

The arithmetic mean length was calculated in the same way:

$$L_{arith.\ mean..} = \Sigma L / \Sigma n \qquad \text{equation (8)}$$

ΣL =sum of all recorded lengths for each time point and fibre type
Σn =sum of all recorded fibres for each time point and fibre type

The geometric mean diameter was calculated as follows:

$$D_{geom.\ mean..} = e^{\Sigma \ln D / \Sigma n} \qquad \text{equation (9)}$$

$\Sigma \ln D$ =sum of the natural logarithm of all recorded diameters for each time point and fibre type
Σn =sum of all recorded fibres for each time point and fibre type

The geometric mean length was calculated in the same way:

$$L_{geom.\ mean..} = e^{\Sigma \ln L / \Sigma n} \qquad \text{equation (10)}$$

$\Sigma \ln L$ =sum of the natural logarithm of all recorded lengths for each time point and fibre type
Σn =sum of all recorded fibres for each time point and fibre type

The length weighted arithmetic mean diameter was calculated as follows:

$$d_{length-weightedarith.mean} = \frac{(D_1 * L_1 + D_2 * L_2 + D_3 * L_3 + ...)}{(L_1 + L_2 + L_3 + ...)} = \frac{\sum (D_i * L_i)}{\sum L_i} \qquad \text{equation (11)}$$

$\Sigma D_i * L_i$ = sum of the product of each recorded diameter and length for each time point and fibre type

ΣL_i = sum of all recorded lengths of fibres for each time point and fibre type

The length weighted geometric mean diameter was calculated as follows:

$$d_{length-weightedgeom.mean} = e^{\frac{(\ln D_1 * L_1 + \ln D_2 * L_2 + \ln D_3 * L_3 + ...)}{(L_1 + L_2 + L_3 + ...)}} = e^{\frac{\Sigma (\ln D_i * L_i)}{\Sigma L_i}} \qquad \text{equation (12)}$$

$\Sigma \ln D_i * L$ = sum of the product of each recorded natural logarithm of diameter and length for each time point and fibre type

ΣL_i = sum of all recorded lengths of fibres for each time point and fibre type

The standard deviation of the geometric mean diameter and length was calculated as follows:

$$S_{geom.mean} = e^{\sqrt{\frac{\sum (\ln D - \ln D_{geom.mean})^2}{n-1}}} \qquad \text{equation (13)}$$

ln D = natural logarithm of diameter or length

$\ln D_{geom.\ mean}$ = natural logarithm of geometric mean diameter or length

n = sum of all recorded fibres

The total length of fibres per lung was calculated as follows:

$$L_{total} = C_{lung} \times L_{mean} \qquad \text{equation (14)}$$

L_{total} = total length of fibres per lung

C_{lung} = number of fibres per lung

L_{mean} = arithmetic mean length

The mass of the fibres per lung was calculated as follows:

$$m_{fibres} = \sum \frac{D_i^2 \times \pi \times L_i}{4} \times \frac{C_{Lung}}{n} \times \delta$$

equation (15)

m_{fibres} = total mass of fibres in the lung
D_i = diameter of the individual fibre recorded
L_i = length of the individual fibre recorded
d = density of the fibre material (g/cm³)
n = sum of all recorded fibres
C_{lung} = number of fibres per lung

Statistical Analysis

Since during fibre counting the probability for non-occurrence of fibres is greater than the probability for occurrence, a Poisson-Distribution is assumed for counting statistics. For large numbers this distribution changes via a t-distribution to a normal-distribution. For the geometric parameters it was assumed that they follow a normal-distribution. Amongst arithmetic means with standard deviations, the geometric means were also calculated in order to avoid overestimation of single large numbers.

For recounts, the corresponding groups were checked for their significance using a t-test.

All given figures were rounded from the first up to the third decimal. The calculations however were done with the original figures. Therefore little differences in the sum may occur if the single values in the tables are recalculated.

Results

Fibre Number Concentrations

Aerosol Samples

The aerosol concentrations are shown in diagram 1.

More detailed information is given in Table A3 (Appended Data Tables).

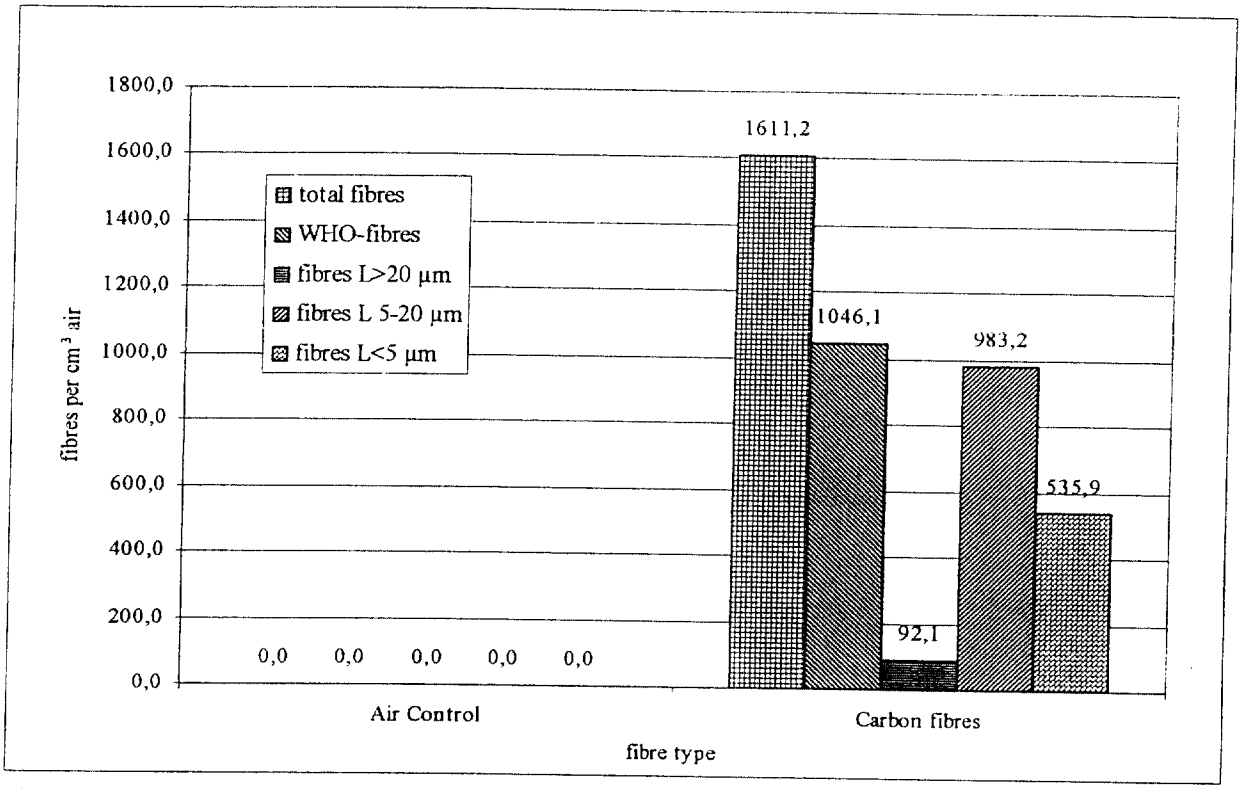


Diagram 1: Aerosol Concentrations of fibres per cm^3 of air, averaged for each design group.

- total fibres means all objects without any limit in length nor diameter but a length to diameter ratio (L:D) ≥ 3.
- WHO-fibres means the fibres with a length (L) > 5 µm, diameter (D) < 3µm and L:D ≥ 3.
- L < 5 µm means all objects with a length less than 5 µm and a length to diameter ratio (L:D) ≥ 3.
- L 5 - 20 µm means all objects with a length equal or greater than 5 µm, but not more than 20 µm, and a length to diameter ratio (L:D) ≥ 3.
- L > 20 µm means all objects with a length greater than 20 µm and a length to diameter ratio (L:D) ≥ 3.

Digested lung samples

Fibre number concentrations in millions of carbon fibres in the digested lung samples for the different sacrifice time points as for different size fractions are shown in the diagram 2. More detailed information are given in Table A5 (Appended Data Tables).

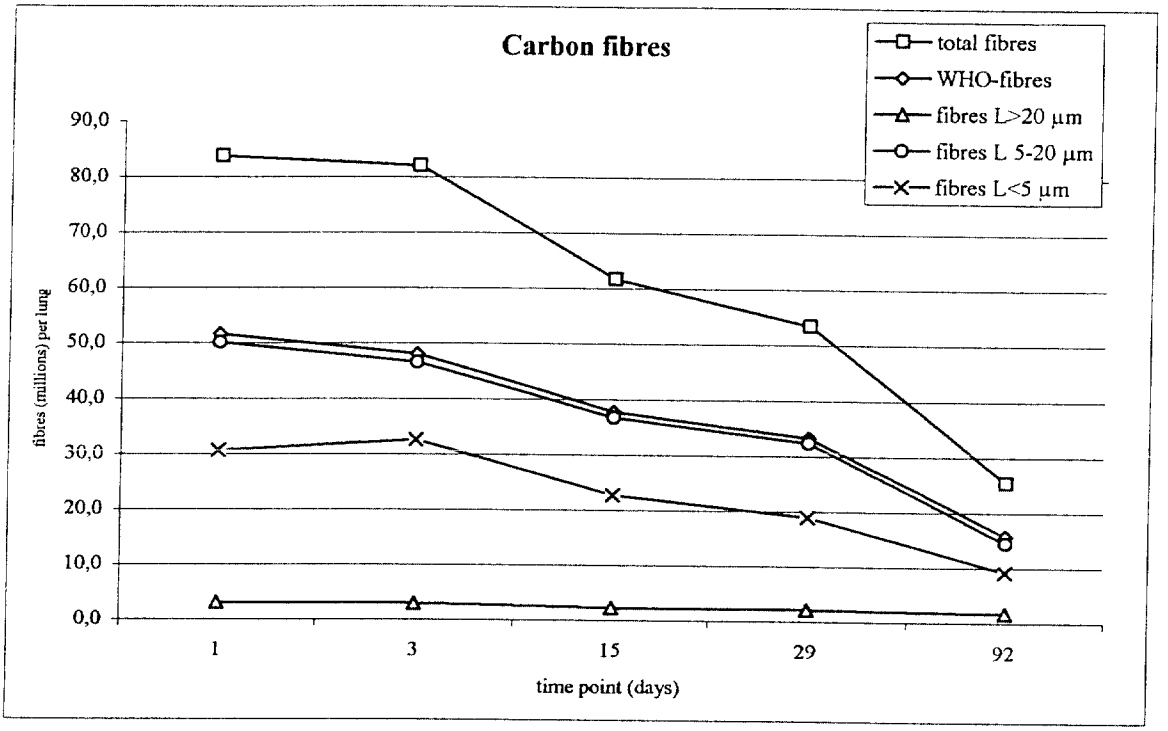


Diagram 2: Fibre number in millions of carbon fibres in the digested lung samples for sacrifice time point 1, 3, 15, 29 and 92 days for different size fractions

Fibre Dimensions

In the diagrams 3 to 10 are shown the geometric means of diameter and length and the aspect ratio for carbon fibres for aerosols and in the digested lung samples for different sacrifice time points.

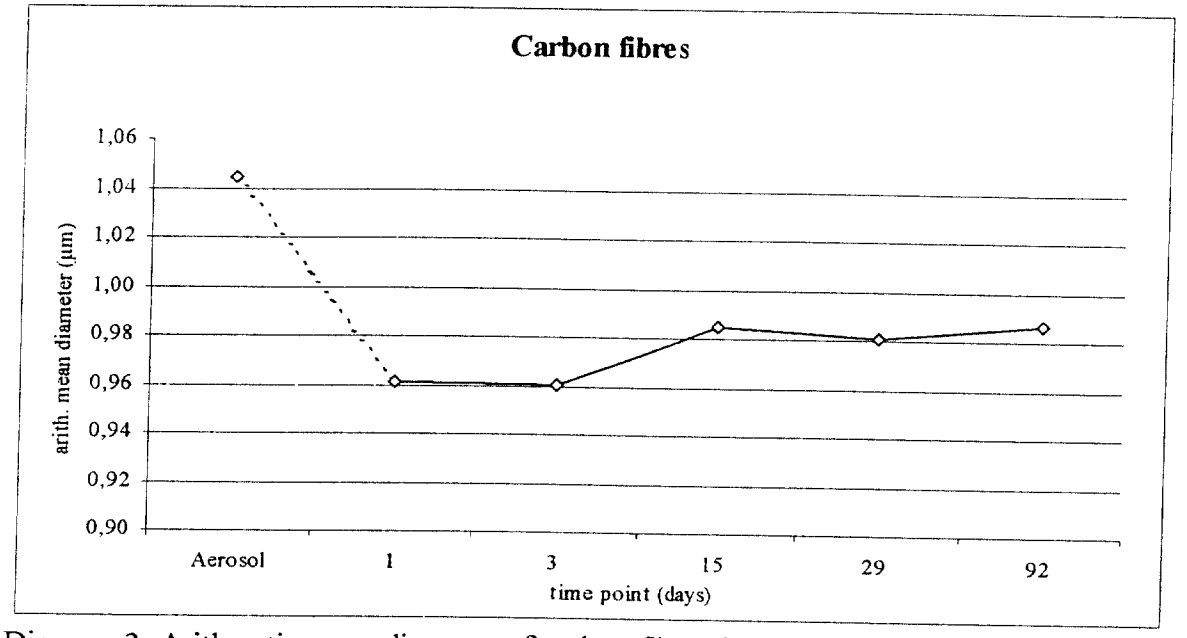


Diagram 3: Arithmetic mean diameter of carbon fibres for aerosol sample and in the digested lung samples for sacrifice time point 1, 3, 15, 29 and 92 days.

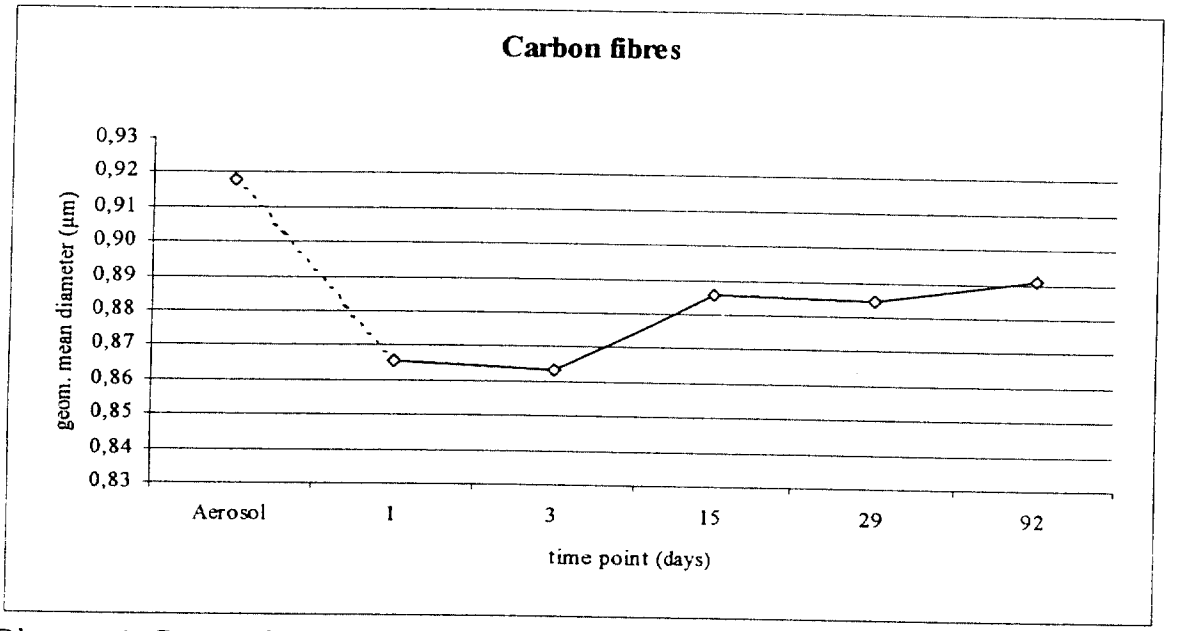


Diagram 4: Geometric mean diameter of carbon fibres for aerosol sample and in the digested lung samples for sacrifice time point 1, 3, 15, 29 and 92 days.

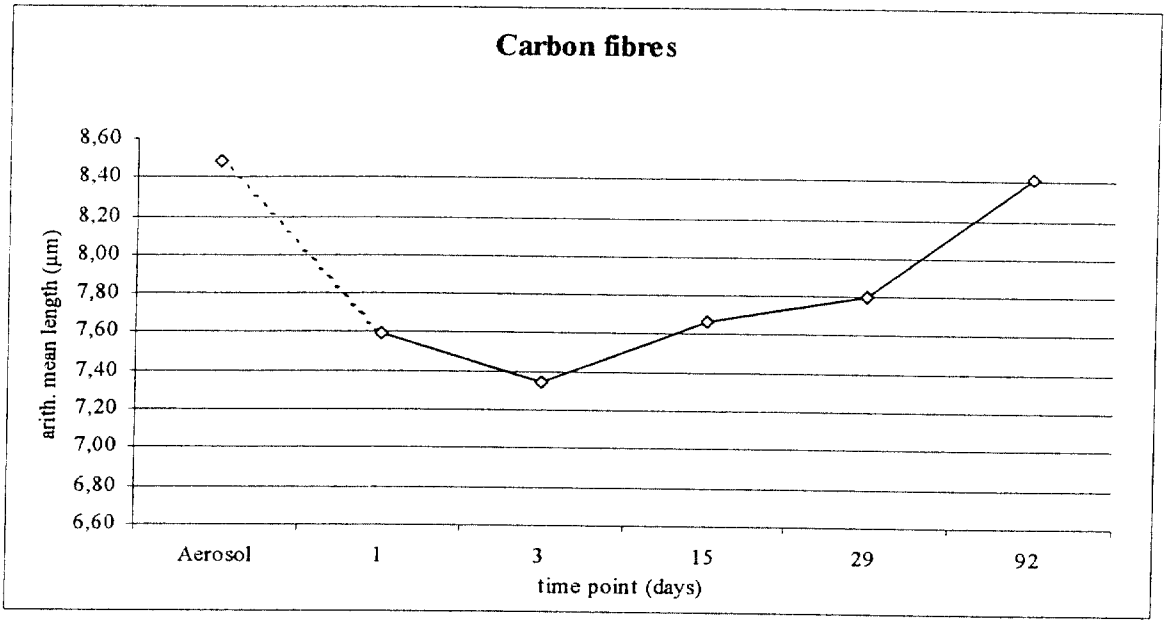


Diagram 5: Arithmetic mean length of carbon fibres for aerosol sample and in the digested lung samples for sacrifice time point 1, 3, 15, 29 and 92 days.

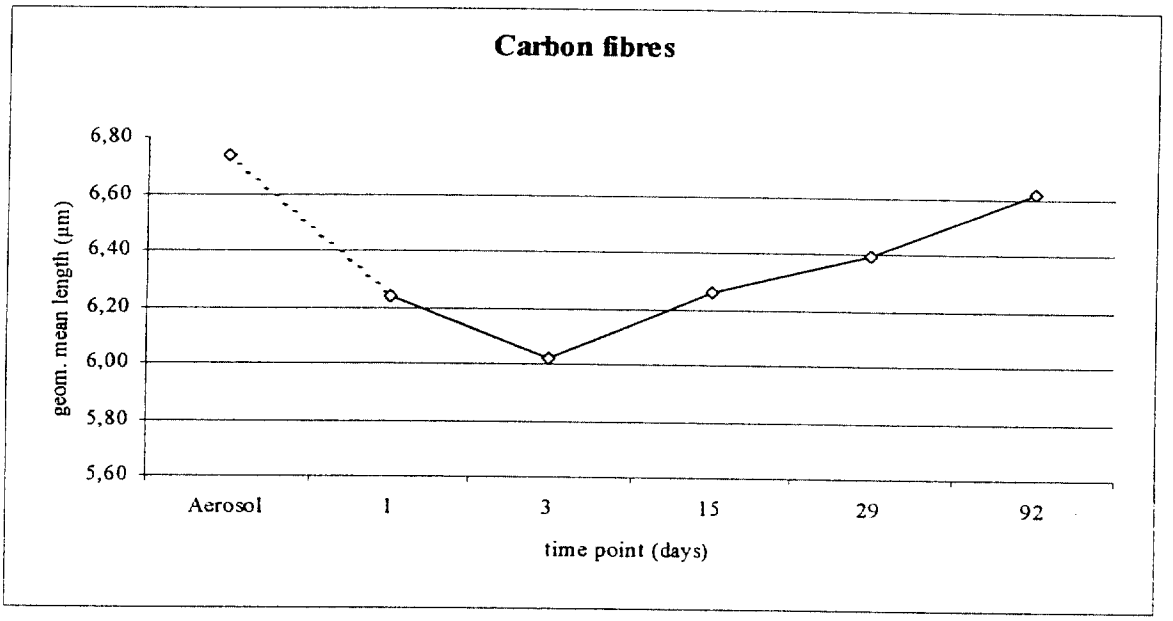


Diagram 6: Geometric mean length of carbon fibres for aerosol sample and in the digested lung samples for sacrifice time point 1, 3, 15, 29 and 92 days.

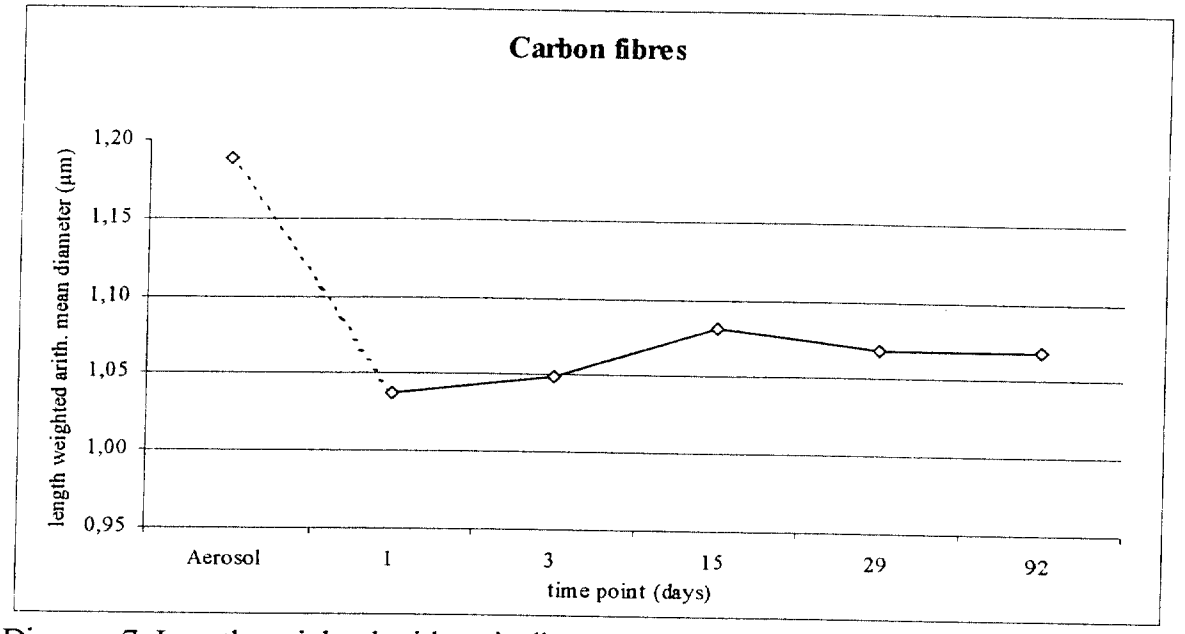


Diagram 7: Length-weighted arithmetic diameter of carbon fibres for aerosol sample and in the digested lung samples for sacrifice time point 1, 3, 15, 29 and 92 days.

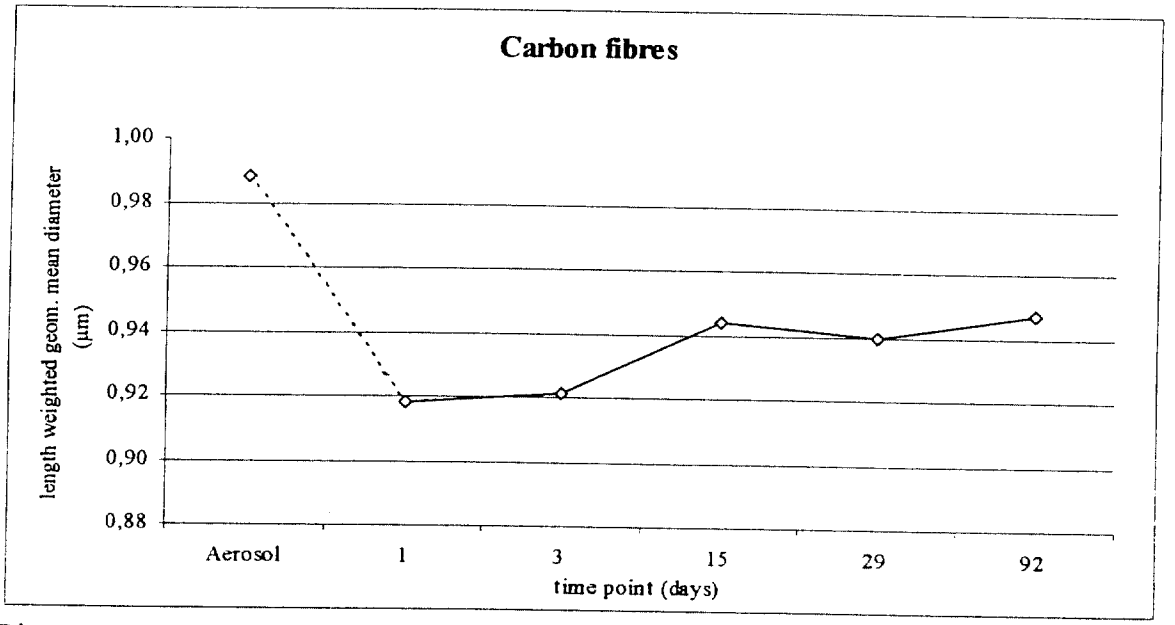


Diagram 8: Length-weighted geometric diameter of carbon fibres for aerosol sample and in the digested lung samples for sacrifice time point 1, 3, 15, 29 and 92 days.

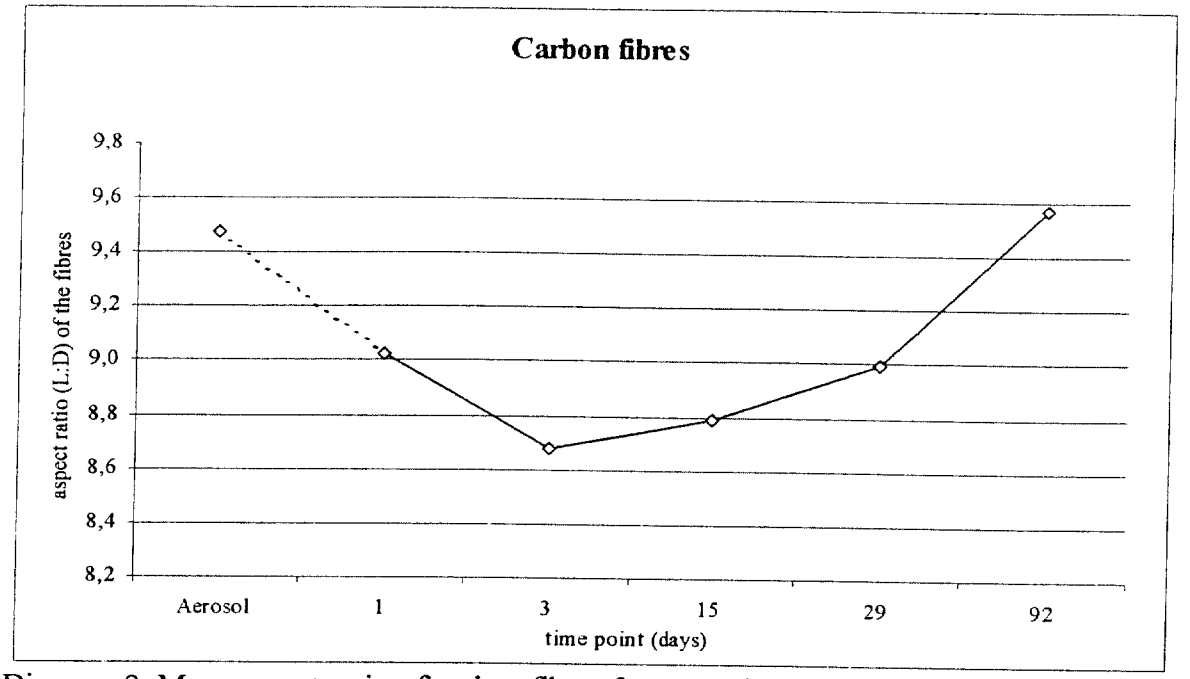


Diagram 9: Mean aspect ratio of carbon fibres for aerosol sample and in the digested lung samples for sacrifice time point 1, 3, 15, 29 and 92 days, considering length and diameter for each individual fibre.

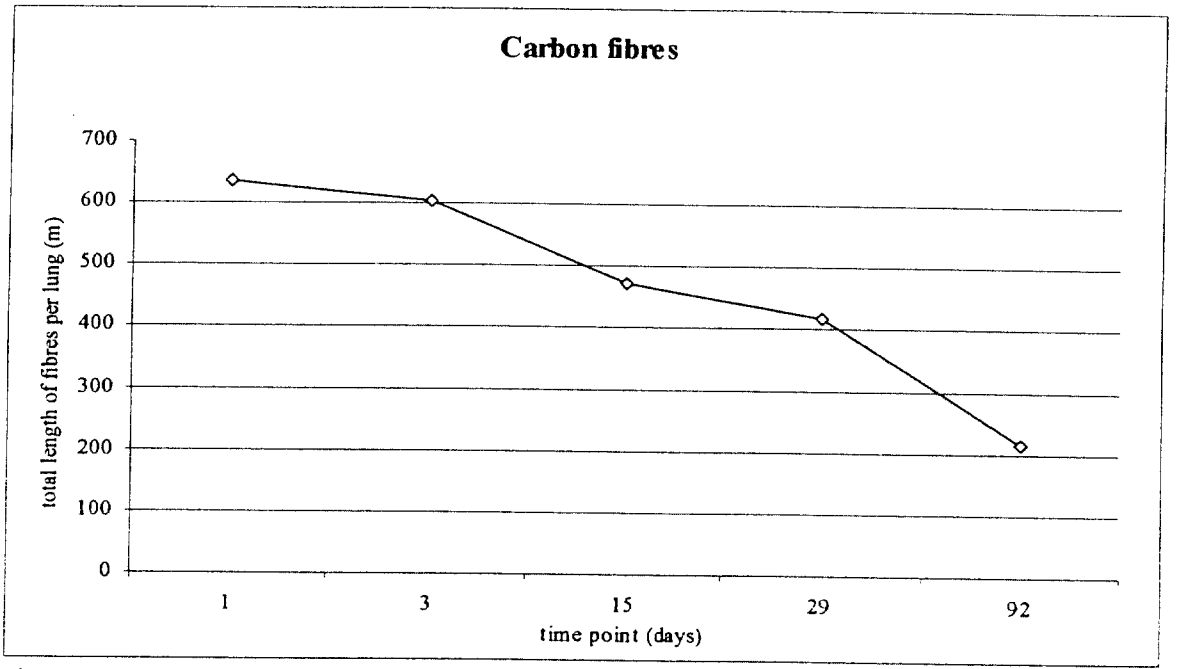


Diagram 10: Total length of fibres per lung of carbon fibres in the digested lung samples for sacrifice time point 1, 3, 15, 29 and 92 days.

More detailed information is given in tables A1 to A5 (Appended Data Tables).

Quality control

In this project about 25 thousand fibres were counted and sized. The thinnest fibre detected had a diameter of 0,1 µm. The longest fibre detected had a length of 110 µm. The average length to diameter ratio was measured in the range between 7,9 and 10,5. In these size determinations several quality assurance audits were carried out in order to verify the results. In each series of filtration the liquids used at GSA were filtered too and handled and evaluated in the SEM in the same way as RCC-samples. Totally 11 of such blank-filters were produced by filtering 20 cm^3 of the liquids through each.

In each case 1 mm^2 was evaluated. On these filters an average of 0,67 fibres/mm^2 with a standard deviation of 0,49 was found (see table 1). Since from the suspensions of the project mostly only 1 cm^3 or less was filtered, it can be assumed that during the handling at GSA the contamination was less than 2 fibres per mm^2.

One fibre detected on a blank in 1 mm^2 means 282 fibres on the whole filter, resp. 282 fibres in 90 cm^3 ***(formula 1+2).*** Since at GSA for a single sample never more than 20 cm^3 of liquids were used, 282 fibres can be assumed as background level for handling the samples at GSA.

To determine the detection limit of the method, the control samples were used. These filters contain the ash of lungs which were not exposed to fibres. ***Table 2*** shows, that the highest fibre loading was 102082 per lung which corresponds to a detection limit of 33853 fibres/lung (Poisson 95%).

Table 1: Blanks

Volume filtered: 20 cm^3
Observed filter area: 1 mm^2

GSA-No.	fibres n WHO	fibres n total	Particles	fibre length min.	fibre length Max.	fibre diameter min.	fibre diameter max.
BL240801	0	0	0				
BL280801	1	1	0	6,5	6,5	1,3	1,3
BL290801	0	1	0	3,3	3,3	1	1
BL300801	0	0	0				
BL040901	0	0	0				
BL050402	1	1	1	5,5	5,5	1,1	1,1
BL080402	0	1	0	4,5	4,5	0,8	0,8
BL090402	1	1	0	5,5	5,5	0,9	0,9
BL220402	0	0	0				
BL230402	0	1	1	4,6	4,6	1	1
BL240402	1	1	0	5,9	5,9	1,1	1,1
BL250402	0	1	0	4,4	4,4	0,8	0,8

Average fibre number WHO:	0,33	±	0,49				
Average total fibre number:	0,67	±	0,49				
Average particle number:	0,17	±	0,39				
Number of blanks counted:	12						
arithm. mean fibre length	5,03						
standard deviation	0,98						
arithm. mean fibre diameter	1,00						
Standard deviation	0,16						
geom. mean fibre length	4,93						
standard deviation	1,23						
geom. mean fibre length	0,99						
standard deviation	1,18						

Table 2: Counting Air-Control Samples

GSA-No.	measure date or animal No.	Group design.	Volume (l)	Area (mm^2)	Fibres counted	Fibres per ml	Fibres per unit (90 ml)	aerosol sampled (cm^3)	Fibres per cm^3 aerosol	Fibres per lung
186500	-	aerosol	0,005	0,5	1	114	10286	299360	0,034	
186501	-	aerosol	0,005	0,5	0	0	0	335056	0,000	
186502	-	aerosol	0,005	0,5	0	0	0	324720	0,000	
186503	-	aerosol	0,005	0,5	1	114	10286	309710	0,033	
186504	-	aerosol	0,005	0,5	0	0	0	295290	0,000	
186505	1	1 day	0,001	0,5	2	1134				102082
186506	2	1 day	0,001	0,5	1	567				51041
186507	3	1 day	0,001	0,5	0	0				0
186508	4	1 day	0,001	0,5	1	571				51429
186509	5	1 day	0,001	0,5	0	0				0
186510	6	29 days	0,001	0,5	1	567				51041
186511	7	29 days	0,001	0,5	1	567				51041
186512	8	29 days	0,001	0,5	1,5	857				77143
186513	9	29 days	0,001	0,5	0	0				0
186514	10	29 days	0,001	0,5	0	0				0
186515	11	92 days	0,001	0,5	2	1134				102082
186516	12	92 days	0,001	0,5	2	1134				102082
186517	13	92 days	0,001	0,5	1	567				51041
186518	14	92 days	0,001	0,5	2	1134				102082
186519	15	92 days	0,001	0,5	1	567				51041

Appended Data Tables

Content

Table A1a: Aerosol Air Control						
sample identification GSA-No.	186500	186501	186502	186503	186504	
sample identification RCC	15.08.2001	16.08.2001	17.08.2001	18.08.2001	19.08.2001	mean
samples evaluated	1	1	1	1	1	5
Number of Fibres evaluated	1	0	0	1	0	2
Number of total fibres per cm³ air	0,034	0,000	0,000	0,033	0,000	0,014
WHO Fibres per cm³ air	0,000	0,000	0,000	0,000	0,000	0,000
Number of WHO-fibres/total fibres in %	0%	N.A.	N.A.	0%	N.A.	0%
Number of WHO-fibres l>20µm per cm³ air	0,000	0,000	0,000	0,000	0,000	0,000
Number of WHO-fibres l>20µm/WHO-fibres in %	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Number of WHO-fibres l>40µm per cm³ air	0,000	0,000	0,000	0,000	0,000	0,000
Number of WHO-fibres l>40µm/WHO-fibres in %	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Number of fibres l >20 µm per cm³ air	0,000	0,000	0,000	0,000	0,000	0,000
Number of fibres l>20 µm/total fibres in %	0%	N.A.	N.A.	0%	N.A.	0%
Number of fibres l >40 µm per cm³ air	0,000	0,000	0,000	0,000	0,000	0,000
Number of fibres l>40 µm/total fibres in %	0,0%	N.A.	N.A.	0,0%	N.A.	0,0%
Number of fibres l 5 - 20 µm per cm³ air	0,000	0,000	0,000	0,000	0,000	0,000
Number of fibres l <5 µm per cm³ air	0,034	0,000	0,000	0,033	0,000	0,014
Diameter Range (µm)	0,90 - 0,9	N.A.	N.A.	1,20 - 1,2	N.A.	0,90 - 1,2
Length Range (µm)	3,2 - 3,2	N.A.	N.A.	4,2 - 4,2	N.A.	3,2 - 4,2
Mean Diameter (µm) total fibres	0,90	N.A.	N.A.	1,20	N.A.	1,05
Std. Dev.	0,00	N.A.	N.A.	0,00	N.A.	0,17
Mean Diameter (µm) fibres l>20 µm	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Std. Dev.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Mean Diameter (µm) WHO-fibres	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Std. Dev.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Mean Length (µm) total fibres	3,20	N.A.	N.A.	4,20	N.A.	3,70
Std. Dev.	0,00	N.A.	N.A.	0,00	N.A.	0,58
Mean Length (µm) fibres l>20 µm	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Std. Dev.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Mean Length (µm) WHO-fibres	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Std. Dev.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
GMD (µm) total fibres	0,90	N.A.	N.A.	1,20	N.A.	1,04
Std. Dev.	1,00	N.A.	N.A.	1,00	N.A.	1,18
GMD (µm) fibres l>20 µm	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Std. Dev.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
GMD (µm) WHO-fibres	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Std. Dev.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
GML (µm) total fibres	3,20	N.A.	N.A.	4,20	N.A.	3,67
Std. Dev.	1,00	N.A.	N.A.	1,00	N.A.	1,17
GML (µm)fibres l>20 µm	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Std. Dev.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
GML (µm) WHO-fibres	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Std. Dev.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Length weighted arthm. diameter (µm)	0,90	N.A.	N.A.	1,20	N.A.	1,07
Length weighted geom. diameter (µm)	0,90	N.A.	N.A.	1,20	N.A.	1,05
Mode diameter (µm)	0,9	N.A.	N.A.	1,2	N.A.	0,9
Mode length (µm)	3,2	N.A.	N.A.	4,2	N.A.	3,2
Median diameter (µm)	0,9	N.A.	N.A.	1,2	N.A.	1,1
Median length (µm)	3,2	N.A.	N.A.	4,2	N.A.	3,7
Aspect ratio	3,6	N.A.	N.A.	3,5	N.A.	3,53
Total length (m) per cm³ air	0,1	0,0	0,0	0,1	0,0	0,1
fibre-mass in mg per cm³ air (density 1,5 g/cm³)	0,000	0,000	0,000	0,000	0,000	0,000
Number of Particles evaluated	1	0	0	1	1	3
Mean Number of Particles per cm³ air	0,034	0,000	0,000	0,033	0,035	0,020
Number of particles/(fibres+particles) in %	50%	N.A.	N.A.	50%	100%	60%
<= 1µm particles per cm³ air	0,000	0,000	0,000	0,000	0,000	0,000
> 1µm - <= 3µm particles per cm³ air	0,034	0,000	0,000	0,033	0,035	0,020
> 3µm particles per cm³ air	0,000	0,000	0,000	0,000	0,000	0,000

Table A1b: Aerosol Carbon fibres						
sample identification GSA-No.	186520	186521	186522	186523	186524	
sample identification RCC	15.08.2001	16.08.2001	17.08.2001	18.08.2001	19.08.2001	mean
samples evaluated	1	1	1	1	1	5
Number of Fibres evaluated	602	642,5	564,5	665,5	630	3104,5
Number of total fibres per cm³ air	1887,880	1954,997	1300,909	1466,003	1446,150	1611,188
WHO Fibres per cm³ air	1271,653	1217,119	934,488	886,651	920,486	1046,079
Number of WHO-fibres/total fibres in %	67%	62%	72%	60%	64%	65%
Number of WHO-fibres l>20µm per cm³ air	131,713	77,591	73,745	68,289	91,819	88,631
Number of WHO-fibres l>20µm/WHO-fibres in %	10%	6%	8%	8%	10%	8%
Number of WHO-fibres l>40µm per cm³ air	9,408	4,564	8,066	7,710	14,921	8,934
Number of WHO-fibres l>40µm/WHO-fibres in %	0,7%	0,4%	0,9%	0,9%	1,6%	0,9%
Number of fibres l >20 µm per cm³ air	131,713	77,591	78,354	71,593	101,001	92,050
Number of fibres l>20 µm/total fibres in %	7%	4%	6%	5%	7%	6%
Number of fibres l >40 µm per cm³ air	9,408	4,564	10,370	8,811	17,216	10,074
Number of fibres l>40 µm/total fibres in %	0,5%	0,2%	0,8%	0,6%	1,2%	0,6%
Number of fibres l 5 - 20 µm per cm³ air	1172,869	1163,870	902,225	835,985	841,292	983,248
Number of fibres l <5 µm per cm³ air	583,298	713,536	320,330	558,425	503,857	535,889
Diameter Range (µm)	0,10 - 3,0	0,18 - 3,7	0,25 - 3,6	0,20 - 5,3	0,20 - 4,9	0,10 - 5,3
Length Range (µm)	1,5 - 90,0	1,3 - 110,0	1,7 - 67,5	1,4 - 65,0	1,4 - 92,0	1,3 - 110,0
Mean Diameter (µm) total fibres	0,97	1,03	1,13	1,01	1,08	1,04
Std. Dev.	0,50	0,52	0,53	0,54	0,58	0,54
Mean Diameter (µm) fibres l>20 µm	1,15	1,22	1,33	1,45	1,46	1,33
Std. Dev.	0,50	0,58	0,73	0,92	0,94	0,77
Mean Diameter (µm) WHO-fibres	1,09	1,19	1,22	1,15	1,20	1,17
Std. Dev.	0,53	0,52	0,51	0,51	0,54	0,52
Mean Length (µm) total fibres	8,67	8,11	9,07	7,82	8,90	8,49
Std. Dev.	7,45	6,85	6,99	6,57	7,97	7,19
Mean Length (µm) fibres l>20 µm	29,40	30,54	30,54	29,60	31,04	30,22
Std. Dev.	11,72	15,45	9,85	10,33	11,95	11,78
Mean Length (µm) WHO-fibres	11,08	10,86	10,84	10,40	11,56	10,95
Std. Dev.	8,00	7,33	6,75	6,72	8,32	7,46
GMD (µm) total fibres	0,84	0,91	1,01	0,89	0,96	0,92
Std. Dev.	1,76	1,69	1,60	1,67	1,66	1,68
GMD (µm) fibres l>20 µm	1,01	1,09	1,17	1,25	1,23	1,15
Std. Dev.	1,75	1,67	1,66	1,70	1,78	1,72
GMD (µm) WHO-fibres	0,95	1,07	1,11	1,04	1,08	1,05
Std. Dev.	1,76	1,63	1,57	1,60	1,62	1,64
GML (µm) total fibres	6,88	6,46	7,47	6,22	6,86	6,74
Std. Dev.	1,91	1,93	1,81	1,91	1,99	1,92
GML (µm)fibres l>20 µm	28,04	28,58	29,34	28,19	29,38	28,72
Std. Dev.	1,32	1,38	1,31	1,35	1,37	1,34
GML (µm) WHO-fibres	9,51	9,60	9,54	9,14	9,86	9,53
Std. Dev.	1,66	1,58	1,60	1,59	1,68	1,62
Length weighted arthm. diameter (µm)	1,07	1,15	1,25	1,19	1,27	1,19
Length weighted geom. diameter (µm)	0,90	0,98	1,07	0,97	1,04	0,99
Mode diameter (µm)	1,3	1,3	1,1	1,2	1,1	1,2
Mode length (µm)	5,7	5,3	7,0	2,7	4,3	4,7
Median diameter (µm)	0,9	1,0	1,0	0,9	1,0	1,0
Median length (µm)	6,3	6,3	7,0	6,1	6,3	6,4
Aspect ratio	11,3	9,2	9,2	8,7	9,2	9,47
Total length (m) per m³ air	16358,5	15848,7	11804,5	11468,9	12863,7	13679,2
fibre-mass in mg per m³ air (density 1,5 g/cm³)	21,583	24,119	21,132	20,152	25,319	22,461
Number of Particles evaluated	101	100	103	102	103	509
Mean Number of Particles per cm³ air	567,943	930,738	807,047	938,418	1196,948	888,219
Number of particles/(fibres+particles) in %	23%	32%	38%	39%	45%	36%
<= 1µm particles per cm³ air	16,870	27,922	23,506	46,001	116,209	46,101
> 1µm - <= 3µm particles per cm³ air	489,218	781,820	579,820	708,414	929,668	697,788
> 3µm particles per cm³ air	61,855	120,996	203,721	184,004	151,071	144,329

Table 2a: Air Control day 1						
sample identification GSA-No.	186505	186506	186507	186508	186509	
sample identification RCC	Animal 1	Animal 2	Animal 3	Animal 4	Animal 5	mean
samples evaluated	1	1	1	1	1	5
Number of Fibres evaluated	2	1	0	1	0	4
Number of total fibres per lung lobes in millions	0,102	0,051	0,000	0,051	0,000	0,041
WHO Fibres per lung lobes in millions	0,051	0,051	0,000	0,000	0,000	0,020
Number of WHO-fibres/total fibres in %	50%	100%	N.A.	0%	N.A.	50%
Number of WHO-fibres l>20µm per lung lobes in millions	0,000	0,000	0,000	0,000	0,000	0,000
Number of WHO-fibres l>20µm/WHO-fibres in %	0%	0%	N.A.	N.A.	N.A.	0%
Number of WHO-fibres l>40µm per lung lobes in millions	0,000	0,000	0,000	0,000	0,000	0,000
Number of WHO-fibres l>40µm/WHO-fibres in %	0,0%	0,0%	N.A.	N.A.	N.A.	0,0%
Number of fibres l >20 µm per lung lobes in millions	0,000	0,000	0,000	0,000	0,000	0,000
Number of fibres l>20 µm/total fibres in %	0%	0%	N.A.	0%	N.A.	0%
Number of fibres l >40 µm per lung lobes in millions	0,000	0,000	0,000	0,000	0,000	0,000
Number of fibres l>40 µm/total fibres in %	0.0%	0,0%	N.A.	0,0%	N.A.	0,0%
Number of fibres l 5 - 20 µm per lung lobes in millions	0,051	0,051	0,000	0,000	0,000	0,020
Number of fibres l <5 µm per lung lobes in millions	0,051	0,000	0,000	0,051	0,000	0,020
Diameter Range (µm)	0,57 - 2,1	2,10 - 2,1	N.A.	0,60 - 0,6	N.A.	0,57 - 2,1
Length Range (µm)	4,2 - 8,1	6,8 - 6,8	N.A.	4,0 - 4,0	N.A.	4,0 - 8,1
Mean Diameter (µm) total fibres	1,34	2,10	N.A.	0,60	N.A.	1,34
Std. Dev.	0,88	0,00	N.A.	0,00	N.A.	0,81
Mean Diameter (µm) fibres l>20 µm	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Std. Dev.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Mean Diameter (µm) WHO-fibres	2,10	2,10	N.A.	N.A.	N.A.	2,10
Std. Dev.	0,00	0,00	N.A.	N.A.	N.A.	0,00
Mean Length (µm) total fibres	6,15	6,80	N.A.	4,00	N.A.	5,78
Std. Dev.	2,25	0,00	N.A.	0,00	N.A.	1,86
Mean Length (µm) fibres l>20 µm	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Std. Dev.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Mean Length (µm) WHO-fibres	8,10	6,80	N.A.	N.A.	N.A.	7,45
Std. Dev.	0,00	0,00	N.A.	N.A.	N.A.	0,75
GMD (µm) total fibres	1,09	2,10	N.A.	0,60	N.A.	1,11
Std. Dev.	2,12	1,00	N.A.	1,00	N.A.	1,98
GMD (µm) fibres l>20 µm	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Std. Dev.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
GMD (µm) WHO-fibres	2,10	2,10	N.A.	N.A.	N.A.	2,10
Std. Dev.	1,00	1,00	N.A.	N.A.	N.A.	1,00
GML (µm) total fibres	5,83	6,80	N.A.	4,00	N.A.	5,52
Std. Dev.	1,46	1,00	N.A.	1,00	N.A.	1,38
GML (µm)fibres l>20 µm	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Std. Dev.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
GML (µm) WHO-fibres	8,10	6,80	N.A.	N.A.	N.A.	7,42
Std. Dev.	1,00	1,00	N.A.	N.A.	N.A.	1,11
Length weighted arthm. diameter (µm)	1,58	2,10	N.A.	0,60	N.A.	1,56
Length weighted geom. diameter (µm)	1,24	2,10	N.A.	0,60	N.A.	1,24
Mode diameter (µm)	0,6	2,1	N.A.	0,6	N.A.	2,1
Mode length (µm)	4,2	6,8	N.A.	4,0	N.A.	4,2
Median diameter (µm)	1,3	2,1	N.A.	0,6	N.A.	1,4
Median length (µm)	6,2	6,8	N.A.	4,0	N.A.	5,5
Aspect ratio	5,6	3,2	N.A.	6,7	N.A.	5,28
Total length (m)	0,6	0,3	0,0	0,2	0,0	0,2
fibre-mass in mg per lung (density 1,5 g/cm^3)	0,002	0,001	0,000	0,001	0,000	0,001
Number of Particles evaluated	4	3	0	0	0	7
Mean Number of Particles per lung lobes in millions	0,204	0,153	0,000	0,000	0,000	0,071
Number of particles/(fibres+particles) in %	67%	75%	N.A.	0%	N.A.	64%
<= 1µm particles per lung lobes in millions	0,000	0,000	0,000	0,000	0,000	0,000
> 1µm - <= 3µm particles per lung lobes in millions	0,102	0,051	0,000	0,000	0,000	0,031
> 3µm particles per lung lobes in millions	0,102	0,102	0,000	0,000	0,000	0,041

Table 2b: Air Control day 29						
sample identification GSA-No.	186510	186511	186512	186513	186514	
sample identification RCC	Animal 6	Animal 7	Animal 8	Animal 9	Animal 10	mean
samples evaluated	1	1	1	1	1	5
Number of Fibres evaluated	1	1	1,5	0	0	3,5
Number of total fibres per lung lobes in millions	0,051	0,051	0,077	0,000	0,000	0,036
WHO Fibres per lung lobes in millions	0,051	0,051	0,077	0,000	0,000	0,036
Number of WHO-fibres/total fibres in %	100%	100%	100%	N.A.	N.A.	100%
Number of WHO-fibres l>20µm per lung lobes in millions	0,000	0,000	0,000	0,000	0,000	0,000
Number of WHO-fibres l>20µm/WHO-fibres in %	0%	0%	0%	N.A.	N.A.	0%
Number of WHO-fibres l>40µm per lung lobes in millions	0,000	0,000	0,000	0,000	0,000	0,000
Number of WHO-fibres l>40µm/WHO-fibres in %	0,0%	0,0%	0,0%	N.A.	N.A.	0,0%
Number of fibres l >20 µm per lung lobes in millions	0,000	0,000	0,000	0,000	0,000	0,000
Number of fibres l>20 µm/total fibres in %	0%	0%	0%	N.A.	N.A.	0%
Number of fibres l >40 µm per lung lobes in millions	0,000	0,000	0,000	0,000	0,000	0,000
Number of fibres l>40 µm/total fibres in %	0,0%	0,0%	0,0%	N.A.	N.A.	0,0%
Number of fibres l 5 - 20 µm per lung lobes in millions	0,051	0,051	0,077	0,000	0,000	0,036
Number of fibres l <5 µm per lung lobes in millions	0,000	0,000	0,000	0,000	0,000	0,000
Diameter Range (µm)	1,50 - 1,5	1,40 - 1,4	1,00 - 1,5	N.A.	N.A.	1,00 - 1,5
Length Range (µm)	11,6 - 11,6	5,1 - 5,1	5,5 - 9,0	N.A.	N.A.	5,1 - 11,6
Mean Diameter (µm) total fibres	1,50	1,40	1,17	N.A.	N.A.	1,33
Std. Dev.	0,00	0,00	0,29	N.A.	N.A.	0,23
Mean Diameter (µm) fibres l>20 µm	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Std. Dev.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Mean Diameter (µm) WHO-fibres	1,50	1,40	1,17	N.A.	N.A.	1,33
Std. Dev.	0,00	0,00	0,29	N.A.	N.A.	0,23
Mean Length (µm) total fibres	11,60	5,10	6,67	N.A.	N.A.	7,63
Std. Dev.	0,00	0,00	2,02	N.A.	N.A.	3,04
Mean Length (µm) fibres l>20 µm	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Std. Dev.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Mean Length (µm) WHO-fibres	11,60	5,10	6,67	N.A.	N.A.	7,63
Std. Dev.	0,00	0,00	2,02	N.A.	N.A.	3,04
GMD (µm) total fibres	1,50	1,40	1,14	N.A.	N.A.	1,31
Std. Dev.	1,00	1,00	1,26	N.A.	N.A.	1,21
GMD (µm) fibres l>20 µm	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Std. Dev.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
GMD (µm) WHO-fibres	1,50	1,40	1,14	N.A.	N.A.	1,31
Std. Dev.	1,00	1,00	1,26	N.A.	N.A.	1,21
GML (µm) total fibres	11,60	5,10	6,48	N.A.	N.A.	7,15
Std. Dev.	1,00	1,00	1,33	N.A.	N.A.	1,47
GML (µm)fibres l>20 µm	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Std. Dev.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
GML (µm) WHO-fibres	11,60	5,10	6,48	N.A.	N.A.	7,15
Std. Dev.	1,00	1,00	1,33	N.A.	N.A.	1,47
Length weighted arthm. diameter (µm)	1,50	1,40	1,23	N.A.	N.A.	1,38
Length weighted geom. diameter (µm)	1,50	1,40	1,17	N.A.	N.A.	1,33
Mode diameter (µm)	1,5	1,4	1,0	N.A.	N.A.	1,5
Mode length (µm)	11,6	5,1	5,5	N.A.	N.A.	11,6
Median diameter (µm)	1,5	1,4	1,0	N.A.	N.A.	1,4
Median length (µm)	11,6	5,1	5,5	N.A.	N.A.	5,5
Aspect ratio	7,7	3,6	5,7	N.A.	N.A.	5,68
Total length (m)	0,6	0,3	0,5	0,0	0,0	0,3
fibre-mass in mg per lung (density 1,5 g/cm³)	0,001	0,000	0,002	0,000	0,000	0,001
Number of Particles evaluated	4	3	0	0	0	7
Mean Number of Particles per lung lobes in millions	0,204	0,153	0,000	0,000	0,000	0,071
Number of particles/(fibres+particles) in %	80%	75%	0%	N.A.	N.A.	67%
<= 1µm particles per lung lobes in millions	0,000	0,000	0,000	0,000	0,000	0,000
> 1µm - <= 3µm particles per lung lobes in millions	0,102	0,000	0,000	0,000	0,000	0,020
> 3µm particles per lung lobes in millions	0,102	0,153	0,000	0,000	0,000	0,051

Table 2c: Air Control day 92						
sample identification GSA-No.	186515	186516	186517	186518	186519	
sample identification RCC	Animal 11	Animal 12	Animal 13	Animal 14	Animal 15	mean
samples evaluated	1	1	1	1	1	5
Number of Fibres evaluated	2	2	1	2	1	8
Number of total fibres per lung lobes in millions	0,102	0,102	0,051	0,102	0,051	0,082
WHO Fibres per lung lobes in millions	0,000	0,051	0,051	0,051	0,000	0,031
Number of WHO-fibres/total fibres in %	0%	50%	100%	50%	0%	38%
Number of WHO-fibres l>20µm per lung lobes in millions	0,000	0,000	0,000	0,000	0,000	0,000
Number of WHO-fibres l>20µm/WHO-fibres in %	N.A.	0%	0%	0%	N.A.	0%
Number of WHO-fibres l>40µm per lung lobes in millions	0,000	0,000	0,000	0,000	0,000	0,000
Number of WHO-fibres l>40µm/WHO-fibres in %	N.A.	0,0%	0%	0%	N.A.	0,0%
Number of fibres l >20 µm per lung lobes in millions	0,000	0,000	0,000	0,000	0,000	0,000
Number of fibres l>20 µm/total fibres in %	0%	0%	0%	0%	0%	0%
Number of fibres l >40 µm per lung lobes in millions	0,000	0,000	0,000	0,000	0,000	0,000
Number of fibres l>40 µm/total fibres in %	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%
Number of fibres l 5 - 20 µm per lung lobes in millions	0,000	0,051	0,051	0,051	0,000	0,031
Number of fibres l <5 µm per lung lobes in millions	0,102	0,051	0,000	0,051	0,051	0,051
Diameter Range (µm)	0,59 - 0,7	0,51 - 2,6	1,00 - 1,0	0,59 - 0,8	0,58 - 0,6	0,51 - 2,6
Length Range (µm)	2,4 - 2,8	2,4 - 19,2	5,5 - 5,5	4,5 - 5,7	2,3 - 2,3	2,3 - 19,2
Mean Diameter (µm) total fibres	0,65	1,56	1,00	0,70	0,58	0,92
Std. Dev.	0,06	1,21	0,00	0,12	0,00	0,67
Mean Diameter (µm) fibres l>20 µm	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Std. Dev.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Mean Diameter (µm) WHO-fibres	N.A.	2,60	100%	80%	N.A.	1,47
Std. Dev.	N.A.	0,00	0%	0%	N.A.	0,88
Mean Length (µm) total fibres	2,60	10,80	5,50	5,10	2,30	5,60
Std. Dev.	0,23	9,70	0,00	0,69	0,00	5,48
Mean Length (µm) fibres l>20 µm	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Std. Dev.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Mean Length (µm) WHO-fibres	N.A.	19,20	550%	570%	N.A.	10,13
Std. Dev.	N.A.	0,00	0%	0%	N.A.	7,02
GMD (µm) total fibres	0,64	1,15	1,00	0,69	0,58	0,79
Std. Dev.	1,10	2,56	1,00	1,19	1,00	1,66
GMD (µm) fibres l>20 µm	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Std. Dev.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
GMD (µm) WHO-fibres	N.A.	2,60	100%	80%	N.A.	1,28
Std. Dev.	N.A.	1,00	100%	100%	N.A.	1,75
GML (µm) total fibres	2,59	6,79	5,50	5,06	2,30	4,22
Std. Dev.	1,09	3,32	1,00	1,15	1,00	2,01
GML (µm)fibres l>20 µm	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
Std. Dev.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
GML (µm) WHO-fibres	N.A.	19,20	550%	570%	N.A.	8,44
Std. Dev.	N.A.	1,00	100%	100%	N.A.	1,89
Length weighted arthm. diameter (µm)	0,64	2,37	1,00	0,71	0,58	1,53
Length weighted geom. diameter (µm)	0,64	1,79	1,00	0,69	0,58	0,98
Mode diameter (µm)	0,7	2,6	1,0	0,8	0,6	0,6
Mode length (µm)	2,4	19,2	5,5	5,7	2,3	2,4
Median diameter (µm)	0,6	1,6	1,0	0,7	0,6	0,6
Median length (µm)	2,6	10,8	5,5	5,1	2,3	3,7
Aspect ratio	4,1	6,0	5,5	7,4	4,0	5,56
Total length (m)	0,3	1,1	0,3	0,5	0,1	0,5
fibre-mass in mg per lung (density 1,5 g/cm^3)	0,000	0,006	0,001	0,003	0,001	0,002
Number of Particles evaluated	4	3	1	0	1	9
Mean Number of Particles per lung lobes in millions	0,204	0,153	0,051	0,000	0,051	0,092
Number of particles/(fibres+particles) in %	67%	60%	50%	0%	50%	53%
< = 1µm particles per lung lobes in millions	0,000	0,000	0,000	0,000	0,000	0,000
> 1µm - <= 3µm particles per lung lobes in millions	0,102	0,000	0,000	0,000	0,000	0,020
> 3µm particles per lung lobes in millions	0,102	0,153	0,051	0,000	0,051	0,071

Table 2d: Carbon fibres day 1								
sample identification GSA-No.	186525	186526	186527	186528	186529	186530	186531	
sample identification RCC	Animal 16	Animal 17	Animal 18	Animal 19	Animal 20	Animal 21	Animal 22	mean
samples evaluated	1	1	1	1	1	1	1	7
Number of Fibres evaluated	714	612	636	653	648	684,5	637,5	4585
Number of total fibres per lung lobes in millions	97,920	82,477	92,354	89,385	88,869	91,614	44,389	83,858
WHO Fibres per lung lobes in millions	55,474	54,581	58,302	55,780	55,337	54,407	27,852	51,676
Number of WHO-fibres/total fibres in %	57%	66%	63%	62%	62%	59%	63%	62%
Number of WHO-fibres l>20µm per lung lobes in	3,771	3,976	3,775	2,601	3,429	1,941	1,671	3,023
Number of WHO-fibres l>20µm/WHO-fibres in %	7%	7%	6%	5%	6%	4%	6%	6%
Number of WHO-fibres l>40µm per lung lobes in	0,069	0,270	0,000	0,068	0,069	0,335	0,139	0,136
Number of WHO-fibres l>40µm/WHO-fibres in %	0,1%	0,5%	0,0%	0,1%	0,1%	0,6%	0,5%	0,3%
Number of fibres l >20 µm per lung lobes in millions	3,771	3,976	3,921	2,601	3,429	1,941	1,706	3,049
Number of fibres l>20 µm/total fibres in %	4%	5%	4%	3%	4%	2%	4%	4%
Number of fibres l >40 µm per lung lobes in millions	0,069	0,270	0,000	0,068	0,069	0,335	0,139	0,136
Number of fibres l>40 µm/total fibres in %	0,1%	0,3%	0,0%	0,1%	0,1%	0,4%	0,3%	0,2%
Number of fibres l 5 - 20 µm per lung lobes in millions	53,486	52,492	55,761	54,685	53,280	53,135	28,096	50,133
Number of fibres l <5 µm per lung lobes in millions	40,663	26,010	32,672	32,099	32,160	36,539	14,587	30,676
Diameter Range (µm)	0,23 - 2,7	0,30 - 3,2	0,23 - 3,5	0,24 - 3,1	0,23 - 3,5	0,20 - 3,2	0,30 - 3,1	0,20 - 3,5
Length Range (µm)	1,3 - 45,0	1,7 - 65,0	1,5 - 39,0	1,3 - 50,0	1,7 - 45,0	1,3 - 57,0	1,5 - 65,0	1,3 - 65,0
Mean Diameter (µm) total fibres	0,96	0,94	1,00	0,94	0,99	0,87	1,04	0,96
Std. Dev.	0,46	0,40	0,46	0,44	0,45	0,41	0,46	0,44
Mean Diameter (µm) fibres l>20 µm	1,22	0,99	1,15	0,97	0,94	0,93	1,10	1,05
Std. Dev.	0,48	0,38	0,59	0,40	0,34	0,34	0,58	0,47
Mean Diameter (µm) WHO-fibres	1,15	1,03	1,11	1,05	1,12	0,97	1,15	1,08
Std. Dev.	0,47	0,41	0,46	0,47	0,46	0,44	0,46	0,46
Mean Length (µm) total fibres	7,26	8,12	7,70	7,47	7,78	7,21	7,76	7,60
Std. Dev.	5,31	5,99	5,53	5,06	5,44	5,31	5,64	5,47
Mean Length (µm) fibres l>20 µm	25,06	27,59	26,38	24,97	25,92	30,17	27,36	26,62
Std. Dev.	4,27	8,55	4,99	6,13	5,02	10,18	8,48	6,98
Mean Length (µm) WHO-fibres	10,28	10,39	10,09	9,83	10,31	9,77	10,06	10,10
Std. Dev.	5,29	6,20	5,58	5,06	5,47	5,52	5,88	5,58
GMD (µm) total fibres	0,86	0,86	0,90	0,84	0,89	0,78	0,94	0,87
Std. Dev.	1,64	1,50	1,61	1,59	1,60	1,61	1,55	1,59
GMD (µm) fibres l>20 µm	1,13	0,92	1,04	0,90	0,87	0,86	0,98	0,96
Std. Dev.	1,47	1,43	1,55	1,46	1,48	1,51	1,57	1,51
GMD (µm) WHO-fibres	1,05	0,95	1,01	0,94	1,02	0,87	1,06	0,98
Std. Dev.	1,55	1,50	1,58	1,60	1,58	1,62	1,52	1,57
GML (µm) total fibres	5,82	6,70	6,31	6,21	6,39	5,93	6,44	6,24
Std. Dev.	1,94	1,82	1,85	1,82	1,85	1,84	1,80	1,85
GML (µm)fibres l>20 µm	24,77	26,66	25,96	24,41	25,50	28,73	26,38	25,92
Std. Dev.	1,16	1,28	1,19	1,22	1,19	1,36	1,29	1,24
GML (µm) WHO-fibres	9,30	9,26	9,05	8,90	9,27	8,83	9,00	9,08
Std. Dev.	1,52	1,56	1,55	1,53	1,55	1,52	1,55	1,54
Length weighted arthm. diameter (µm)	1,09	0,99	1,09	1,00	1,06	0,92	1,11	1,04
Length weighted geom. diameter (µm)	0,94	0,90	0,95	0,89	0,95	0,82	0,99	0,92
Mode diameter (µm)	1,0	0,6	1,3	1,1	1,3	1,1	1,0	1,0
Mode length (µm)	4,3	3,5	4,3	3,5	4,7	3,7	4,0	4,0
Median diameter (µm)	0,9	0,9	1,0	0,9	0,9	0,8	1,0	0,9
Median length (µm)	5,8	6,5	6,2	6,0	6,3	5,8	6,4	6,1
Aspect ratio	8,3	9,6	8,8	9,2	9,0	9,8	8,4	9,02
Total length (m)	710,7	669,6	710,9	667,9	691,0	660,4	344,4	637,3
fibre-mass in mg per lung (density 1,5 g/cm^3)	0,929	0,712	0,936	0,752	0,864	0,635	0,474	0,758
Number of Particles evaluated	106,5	100	104,5	101	105,5	104	101	722,5
Mean Number of Particles per lung lobes in millions	58,423	30,947	46,903	40,554	47,352	41,759	29,100	42,148
Number of particles/(fibres+particles) in %	37%	27%	34%	31%	35%	31%	40%	33%
<= 1µm particles per lung lobes in millions	2,194	0,619	1,346	1,606	0,449	4,417	0,576	1,601
> 1µm - <= 3µm particles per lung lobes in millions	49,646	25,840	40,395	34,933	42,415	34,130	21,609	35,567
> 3µm particles per lung lobes in millions	6,583	4,487	5,162	4,015	4,488	3,212	6,915	4,980

Table 2e: Carbon fibres day 3								
sample identification GSA-No.	186532	186533	186534	186535	186536	186537	186538	
sample identification RCC	Animal 23	Animal 24	Animal 25	Animal 26	Animal 27	Animal 28	Animal 29	mean
samples evaluated	1	1	1	1	1	1	1	7
Number of Fibres evaluated	735,5	751	697,5	649,5	627,5	627,5	732	4820,5
Number of total fibres per lung lobes in millions	92,288	95,072	81,992	78,212	80,412	69,988	76,893	82,122
WHO Fibres per lung lobes in millions	50,755	51,080	47,314	48,408	51,515	45,004	42,438	48,074
Number of WHO-fibres/total fibres in %	55%	54%	58%	62%	64%	64%	55%	59%
Number of WHO-fibres l>20µm per lung lobes in	2,635	2,342	3,938	2,830	3,076	2,454	3,309	2,940
Number of WHO-fibres l>20µm/WHO-fibres in %	5%	5%	8%	6%	6%	5%	8%	6%
Number of WHO-fibres l>40µm per lung lobes in	0,000	0,063	0,059	0,120	0,256	0,112	0,210	0,117
Number of WHO-fibres l>40µm/WHO-fibres in %	0,0%	0,1%	0,1%	0,2%	0,5%	0,2%	0,5%	0,2%
Number of fibres l >20 µm per lung lobes in millions	2,635	2,342	3,938	2,830	3,076	2,454	3,309	2,940
Number of fibres l>20 µm/total fibres in %	3%	2%	5%	4%	4%	4%	4%	4%
Number of fibres l >40 µm per lung lobes in millions	0,000	0,063	0,059	0,120	0,256	0,112	0,210	0,117
Number of fibres l>40 µm/total fibres in %	0,0%	0,1%	0,1%	0,2%	0,3%	0,2%	0,3%	0,1%
Number of fibres l 5 - 20 µm per lung lobes in millions	49,626	50,384	45,022	47,385	49,144	43,666	40,968	46,599
Number of fibres l <5 µm per lung lobes in millions	40,027	42,345	33,032	27,997	28,192	23,868	32,617	32,583
Diameter Range (µm)	0,22 - 2,9	0,22 - 2,9	0,23 - 3,0	0,20 - 2,8	0,12 - 3,4	0,25 - 3,4	0,20 - 3,0	0,12 - 3,4
Length Range (µm)	1,0 - 39,6	1,5 - 41,0	1,5 - 43,0	1,3 - 42,0	1,3 - 45,0	1,4 - 50,0	1,3 - 55,0	1,0 - 55,0
Mean Diameter (µm) total fibres	0,90	0,92	0,99	0,97	0,95	0,99	1,01	0,96
Std. Dev.	0,41	0,42	0,47	0,46	0,43	0,45	0,47	0,44
Mean Diameter (µm) fibres l>20 µm	0,81	1,21	1,12	1,12	1,00	1,02	1,14	1,07
Std. Dev.	0,33	0,43	0,47	0,48	0,34	0,47	0,40	0,44
Mean Diameter (µm) WHO-fibres	1,04	1,09	1,17	1,10	1,05	1,10	1,19	1,10
Std. Dev.	0,44	0,44	0,46	0,48	0,47	0,46	0,49	0,47
Mean Length (µm) total fibres	6,96	6,70	7,56	7,52	7,88	7,54	7,39	7,34
Std. Dev.	4,88	4,78	5,67	5,23	5,61	5,16	5,90	5,34
Mean Length (µm) fibres l>20 µm	25,24	26,36	25,31	25,36	26,86	25,82	27,83	26,15
Std. Dev.	5,24	5,36	4,56	5,29	7,26	5,74	7,26	5,95
Mean Length (µm) WHO-fibres	9,76	9,50	10,53	9,96	10,25	9,71	10,49	10,03
Std. Dev.	5,03	4,99	5,82	5,30	5,73	5,24	6,37	5,52
GMD (µm) total fibres	0,81	0,84	0,89	0,86	0,86	0,90	0,90	0,86
Std. Dev.	1,59	1,58	1,64	1,65	1,59	1,57	1,62	1,61
GMD (µm) fibres l>20 µm	0,73	1,15	1,03	1,02	0,94	0,91	1,08	0,98
Std. Dev.	1,58	1,41	1,51	1,59	1,44	1,61	1,40	1,53
GMD (µm) WHO-fibres	0,95	1,00	1,08	0,99	0,94	1,00	1,09	1,00
Std. Dev.	1,58	1,52	1,52	1,60	1,61	1,54	1,55	1,56
GML (µm) total fibres	5,77	5,56	6,06	6,21	6,50	6,30	5,93	6,02
Std. Dev.	1,82	1,80	1,91	1,83	1,83	1,80	1,89	1,84
GML (µm)fibres l>20 µm	24,78	25,89	24,95	24,91	26,03	25,34	27,10	25,60
Std. Dev.	1,21	1,21	1,18	1,20	1,28	1,20	1,25	1,22
GML (µm) WHO-fibres	8,86	8,63	9,36	8,98	9,18	8,76	9,28	9,00
Std. Dev.	1,51	1,50	1,58	1,54	1,56	1,53	1,58	1,55
Length weighted arthm. diameter (µm)	0,97	1,03	1,10	1,06	1,01	1,06	1,11	1,05
Length weighted geom. diameter (µm)	0,86	0,90	0,96	0,92	0,90	0,94	0,97	0,92
Mode diameter (µm)	1,2	1,2	1,2	1,1	1,2	1,1	1,1	1,2
Mode length (µm)	3,3	4,3	4,7	3,3	4,3	6,0	4,0	4,7
Median diameter (µm)	0,9	0,9	0,9	0,9	0,9	0,9	0,9	0,9
Median length (µm)	5,5	5,3	5,8	6,0	6,3	6,1	5,7	5,8
Aspect ratio	8,9	7,9	8,5	9,0	9,6	8,7	8,2	8,68
Total length (m)	642,4	637,2	6,0	5,6	5,4	5,4	6,3	603,1
fibre-mass in mg per lung (density 1,5 g/cm^3)	0,7	0,7	0,8	0,7	0,7	0,7	0,8	0,7
Number of Particles evaluated	107	103,5	105	103	101	101	104,5	725
Mean Number of Particles per lung lobes in millions	71,605	63,874	51,840	39,117	55,301	60,831	42,994	55,080
Number of particles/(fibres+particles) in %	44%	40%	39%	33%	41%	47%	36%	40%
<= 1µm particles per lung lobes in millions	10,707	0,000	0,000	0,000	2,738	8,432	0,411	3,184
> 1µm - <= 3µm particles per lung lobes in millions	54,206	60,171	46,409	35,320	46,540	45,171	37,851	46,524
> 3µm particles per lung lobes in millions	6,692	3,703	5,431	3,798	6,023	7,227	4,731	5,372

Table 2f: Carbon fibres day 15								
sample identification GSA-No.	186539	186540	186541	186542	186543	186544	186545	
sample identification RCC	Animal 30	Animal 31	Animal 32	Animal 33	Animal 34	Animal 35	Animal 36	mean
samples evaluated	1	1	1	1	1	1	1	7
Number of Fibres evaluated	683	699	598,5	668	615,5	676	652	4592
Number of total fibres per lung lobes in millions	63,624	66,825	53,200	73,289	56,168	53,794	65,448	61,764
WHO Fibres per lung lobes in millions	37,494	38,336	35,867	44,160	36,593	32,069	40,253	37,825
Number of WHO-fibres/total fibres in %	59%	57%	67%	60%	65%	60%	62%	61%
Number of WHO-fibres l>20µm per lung lobes in	2,608	1,482	2,889	2,688	2,738	1,711	2,208	2,332
Number of WHO-fibres l>20µm/WHO-fibres in %	7%	4%	8%	6%	7%	5%	5%	6%
Number of WHO-fibres l>40µm per lung lobes in	0,279	0,096	0,222	0,439	0,046	0,040	0,000	0,160
Number of WHO-fibres l>40µm/WHO-fibres in %	0,7%	0,2%	0,6%	1,0%	0,1%	0,1%	0,0%	0,4%
Number of fibres l >20 µm per lung lobes in millions	2,608	1,482	2,889	2,688	2,738	1,711	2,208	2,332
Number of fibres l>20 µm/total fibres in %	4%	2%	5%	4%	5%	3%	3%	4%
Number of fibres l >40 µm per lung lobes in millions	0,279	0,096	0,222	0,439	0,046	0,040	0,000	0,160
Number of fibres l>40 µm/total fibres in %	0,4%	0,1%	0,4%	0,6%	0,1%	0,1%	0,0%	0,3%
Number of fibres l 5 - 20 µm per lung lobes in millions	35,445	37,858	34,711	42,569	34,723	32,507	39,199	36,716
Number of fibres l <5 µm per lung lobes in millions	25,571	27,485	15,600	28,032	18,707	19,576	24,041	22,716
Diameter Range (µm)	0,13 - 2,4	0,22 - 2,7	0,25 - 4,1	0,10 - 3,3	0,23 - 4,5	0,30 - 3,2	0,18 - 3,5	0,10 - 4,5
Length Range (µm)	1,3 - 50,0	1,3 - 41,0	1,5 - 75,0	1,5 - 53,0	1,5 - 44,0	1,3 - 63,0	1,5 - 37,5	1,3 - 75,0
Mean Diameter (µm) total fibres	0,93	0,93	1,02	1,03	1,02	1,02	0,96	0,99
Std. Dev.	0,42	0,44	0,47	0,49	0,50	0,46	0,43	0,46
Mean Diameter (µm) fibres l>20 µm	0,95	1,20	1,32	1,16	0,99	1,14	1,16	1,13
Std. Dev.	0,38	0,43	0,52	0,43	0,41	0,46	0,49	0,46
Mean Diameter (µm) WHO-fibres	1,07	1,08	1,13	1,19	1,13	1,15	1,08	1,12
Std. Dev.	0,44	0,48	0,49	0,50	0,50	0,48	0,44	0,48
Mean Length (µm) total fibres	7,37	6,99	8,52	7,68	7,93	7,76	7,54	7,66
Std. Dev.	5,68	4,86	6,54	6,10	5,57	5,53	5,24	5,67
Mean Length (µm) fibres l>20 µm	26,93	26,33	29,31	29,48	24,97	28,06	25,63	27,32
Std. Dev.	7,38	5,82	8,76	9,33	4,87	7,56	4,62	7,36
Mean Length (µm) WHO-fibres	10,12	9,60	10,77	10,47	10,22	10,45	9,99	10,23
Std. Dev.	5,97	4,95	6,87	6,43	5,59	5,69	5,32	5,87
GMD (µm) total fibres	0,84	0,84	0,92	0,91	0,91	0,93	0,87	0,89
Std. Dev.	1,62	1,60	1,56	1,65	1,64	1,53	1,57	1,60
GMD (µm) fibres l>20 µm	0,88	1,13	1,22	1,08	0,91	1,05	1,07	1,04
Std. Dev.	1,48	1,43	1,51	1,45	1,51	1,56	1,50	1,51
GMD (µm) WHO-fibres	0,98	0,98	1,02	1,08	1,02	1,05	0,99	1,02
Std. Dev.	1,58	1,59	1,56	1,60	1,62	1,53	1,52	1,57
GML (µm) total fibres	5,95	5,79	6,99	6,13	6,51	6,41	6,23	6,26
Std. Dev.	1,88	1,82	1,82	1,92	1,85	1,82	1,82	1,85
GML (µm)fibres l>20 µm	26,14	25,77	28,31	28,24	24,58	27,29	25,26	26,53
Std. Dev.	1,26	1,23	1,29	1,33	1,19	1,26	1,19	1,26
GML (µm) WHO-fibres	9,02	8,72	9,46	9,25	9,13	9,43	8,96	9,14
Std. Dev.	1,56	1,51	1,60	1,59	1,57	1,53	1,56	1,56
Length weighted arthm. diameter (µm)	1,01	1,04	1,12	1,16	1,09	1,10	1,05	1,08
Length weighted geom. diameter (µm)	0,90	0,90	0,97	0,99	0,96	0,97	0,92	0,94
Mode diameter (µm)	1,0	1,2	0,7	1,3	1,2	0,7	1,1	1,0
Mode length (µm)	3,3	3,3	6,0	4,3	4,5	4,0	3,3	4,5
Median diameter (µm)	0,9	0,9	0,9	1,0	0,9	0,9	0,9	0,9
Median length (µm)	5,8	5,6	6,5	6,0	6,2	6,1	5,8	6,0
Aspect ratio	9,1	8,3	9,3	8,4	9,2	8,5	8,8	8,79
Total length (m)	468,6	466,9	453,2	562,8	445,5	417,5	493,6	473,3
fibre-mass in mg per lung (density 1,5 g/cm^3)	0,5	0,6	0,6	0,8	0,6	0,6	0,6	0,6
Number of Particles evaluated	104,5	102	101	102,5	101	102,5	100	713,5
Mean Number of Particles per lung lobes in millions	36,852	61,433	28,130	33,737	38,019	27,627	60,229	40,861
Number of particles/(fibres+particles) in %	37%	48%	35%	32%	40%	34%	48%	40%
<= 1µm particles per lung lobes in millions	0,000	4,818	1,114	0,987	3,011	1,348	3,011	2,041
> 1µm - <= 3µm particles per lung lobes in millions	32,620	49,990	20,610	29,787	29,738	22,371	54,206	34,189
> 3µm particles per lung lobes in millions	4,232	6,625	6,406	2,962	5,270	3,908	3,011	4,631

Table 2g: Carbon fibres day 29								
sample identification GSA-No.	186546	186547	186548	186549	186550	186551	186552	
sample identification RCC	Animal 37	Animal 38	Animal 39	Animal 40	Animal 41	Animal 42	Animal 43	mean
samples evaluated	1	1	1	1	1	1	1	7
Number of Fibres evaluated	656	664,5	601	681	654	640,5	630	4527
Number of total fibres per lung lobes in millions	41,589	52,380	52,460	71,536	50,598	59,665	46,273	53,500
WHO Fibres per lung lobes in millions	25,518	31,570	34,959	42,438	31,256	37,401	29,674	33,259
Number of WHO-fibres/total fibres in %	61%	60%	67%	59%	62%	63%	64%	62%
Number of WHO-fibres l>20µm per lung lobes in	1,775	1,655	2,357	3,309	2,128	2,608	1,506	2,191
Number of WHO-fibres l>20µm/WHO-fibres in %	7%	5%	7%	8%	7%	7%	5%	7%
Number of WHO-fibres l>40µm per lung lobes in	0,095	0,079	0,262	0,000	0,000	0,047	0,073	0,079
Number of WHO-fibres l>40µm/WHO-fibres in %	0,4%	0,2%	0,7%	0,0%	0,0%	0,1%	0,2%	0,2%
Number of fibres l >20 µm per lung lobes in millions	1,775	1,655	2,357	3,309	2,128	2,608	1,506	2,191
Number of fibres l>20 µm/total fibres in %	4%	3%	4%	5%	4%	4%	3%	4%
Number of fibres l >40 µm per lung lobes in millions	0,095	0,079	0,262	0,000	0,000	0,047	0,073	0,079
Number of fibres l>40 µm/total fibres in %	0,2%	0,2%	0,5%	0,0%	0,0%	0,1%	0,2%	0,1%
Number of fibres l 5 - 20 µm per lung lobes in millions	24,599	31,333	33,257	40,443	31,720	36,423	28,866	32,377
Number of fibres l <5 µm per lung lobes in millions	15,216	19,391	16,847	27,785	16,750	20,634	15,902	18,932
Diameter Range (µm)	0,22 - 2,8	0,30 - 3,2	0,21 - 3,5	0,10 - 2,7	0,25 - 3,0	0,23 - 3,0	0,19 - 3,3	0,10 - 3,5
Length Range (µm)	1,2 - 57,0	1,6 - 70,0	1,5 - 59,0	1,5 - 36,5	1,0 - 40,0	1,7 - 42,0	1,5 - 46,0	1,0 - 70,0
Mean Diameter (µm) total fibres	0,97	0,97	1,02	0,91	1,02	1,01	0,97	0,98
Std. Dev.	0,45	0,43	0,45	0,43	0,46	0,47	0,45	0,45
Mean Diameter (µm) fibres l>20 µm	1,10	0,97	1,08	0,99	1,22	1,08	1,00	1,07
Std. Dev.	0,50	0,32	0,45	0,40	0,38	0,51	0,32	0,43
Mean Diameter (µm) WHO-fibres	1,11	1,10	1,12	1,05	1,16	1,15	1,08	1,11
Std. Dev.	0,48	0,44	0,44	0,45	0,48	0,48	0,48	0,47
Mean Length (µm) total fibres	7,61	7,70	8,15	7,89	7,81	7,83	7,62	7,80
Std. Dev.	5,80	5,70	6,09	5,91	5,48	5,52	5,17	5,68
Mean Length (µm) fibres l>20 µm	26,89	28,17	27,64	26,56	26,39	25,92	25,65	26,73
Std. Dev.	7,29	10,02	10,12	4,74	4,86	5,11	6,16	7,07
Mean Length (µm) WHO-fibres	10,17	10,34	10,32	10,90	10,29	10,32	9,84	10,31
Std. Dev.	6,10	5,98	6,37	6,00	5,67	5,57	5,22	5,86
GMD (µm) total fibres	0,87	0,88	0,92	0,81	0,93	0,91	0,88	0,88
Std. Dev.	1,60	1,54	1,56	1,66	1,55	1,61	1,58	1,59
GMD (µm) fibres l>20 µm	0,98	0,92	1,00	0,93	1,16	0,98	0,94	0,99
Std. Dev.	1,66	1,38	1,48	1,42	1,42	1,55	1,42	1,49
GMD (µm) WHO-fibres	1,00	1,01	1,04	0,95	1,06	1,05	0,98	1,01
Std. Dev.	1,60	1,50	1,51	1,63	1,54	1,56	1,60	1,56
GML (µm) total fibres	6,17	6,33	6,73	6,29	6,49	6,42	6,37	6,39
Std. Dev.	1,87	1,83	1,81	1,93	1,80	1,86	1,79	1,85
GML (µm)fibres l>20 µm	26,18	26,97	26,28	26,16	25,99	25,49	25,09	26,02
Std. Dev.	1,24	1,32	1,35	1,19	1,19	1,20	1,22	1,24
GML (µm) WHO-fibres	8,97	9,24	9,13	9,68	9,20	9,24	8,88	9,19
Std. Dev.	1,60	1,56	1,58	1,59	1,56	1,56	1,53	1,57
Length weighted arthm. diameter (µm)	1,06	1,05	1,10	0,99	1,12	1,11	1,05	1,07
Length weighted geom. diameter (µm)	0,93	0,93	0,98	0,87	0,99	0,97	0,92	0,94
Mode diameter (µm)	1,2	1,0	1,1	1,3	0,7	1,3	1,1	1,0
Mode length (µm)	5,9	4,0	4,9	3,0	5,0	3,3	3,3	4,0
Median diameter (µm)	0,9	0,9	1,0	0,9	0,9	1,0	0,9	0,9
Median length (µm)	5,8	6,0	6,4	6,0	6,1	6,0	6,2	6,0
Aspect ratio	9,0	8,7	9,0	10,2	8,3	8,7	9,0	9,00
Total length (m)	316,6	403,6	427,7	564,4	395,4	467,4	352,6	417,4
fibre-mass in mg per lung (density 1,5 g/cm^3)	0,4	0,5	0,6	0,6	0,5	0,6	0,4	0,5
Number of Particles evaluated	102	100,5	101	104	100	102	102	711,5
Mean Number of Particles per lung lobes in millions	34,130	32,297	50,692	32,091	33,422	35,971	38,396	36,714
Number of particles/(fibres+particles) in %	45%	38%	49%	31%	40%	38%	45%	41%
<= 1µm particles per lung lobes in millions	3,346	1,285	2,510	0,309	3,008	0,353	2,259	1,867
> 1µm - <= 3µm particles per lung lobes in millions	25,430	24,906	40,654	26,074	23,396	30,857	33,126	29,206
> 3µm particles per lung lobes in millions	5,354	6,106	7,529	5,709	7,019	4,761	3,011	5,641

Table 2h: Carbon fibres day 92								
sample identification GSA-No.	186553	186554	186555	186556	186557	186558	186559	
sample identification RCC	Animal 44	Animal 45	Animal 46	Animal 47	Animal 48	Animal 49	Animal 50	mean
samples evaluated	1	1	1	1	1	1	1	7
Number of Fibres evaluated	685,5	502	415,5	430,5	486,5	588	389,5	3497,5
Number of total fibres per lung lobes in millions	34,891	25,551	21,041	21,789	24,623	29,928	19,825	25,378
WHO Fibres per lung lobes in millions	19,061	17,127	13,014	13,918	15,892	18,043	12,877	15,705
Number of WHO-fibres/total fibres in %	55%	67%	62%	64%	65%	60%	65%	62%
Number of WHO-fibres l>20µm per lung lobes in	1,603	2,290	1,291	1,594	2,227	1,196	1,934	1,734
Number of WHO-fibres l>20µm/WHO-fibres in %	8%	13%	10%	11%	14%	7%	15%	11%
Number of WHO-fibres l>40µm per lung lobes in	0,076	0,051	0,076	0,051	0,076	0,051	0,127	0,073
Number of WHO-fibres l>40µm/WHO-fibres in %	0,4%	0,3%	0,6%	0,4%	0,5%	0,3%	1,0%	0,5%
Number of fibres l >20 µm per lung lobes in millions	1,603	2,290	1,291	1,594	2,227	1,196	1,934	1,734
Number of fibres l>20 µm/total fibres in %	5%	9%	6%	7%	9%	4%	10%	7%
Number of fibres l >40 µm per lung lobes in millions	0,076	0,051	0,076	0,051	0,076	0,051	0,127	0,073
Number of fibres l>40 µm/total fibres in %	0,2%	0,2%	0,4%	0,2%	0,3%	0,2%	0,6%	0,3%
Number of fibres l 5 - 20 µm per lung lobes in millions	17,993	15,091	12,685	12,780	13,944	17,305	11,350	14,450
Number of fibres l <5 µm per lung lobes in millions	15,295	8,169	7,064	7,415	8,452	11,427	6,540	9,195
Diameter Range (µm)	0,25 - 2,7	0,25 - 2,6	0,24 - 2,3	0,14 - 2,4	0,10 - 3,5	0,20 - 3,3	0,25 - 3,6	0,10 - 3,6
Length Range (µm)	1,5 - 48,0	1,7 - 54,0	1,2 - 55,0	1,5 - 49,0	1,5 - 50,0	1,5 - 70,0	1,5 - 47,0	1,2 - 70,0
Mean Diameter (µm) total fibres	0,93	1,00	0,97	0,96	0,98	1,03	1,06	0,99
Std. Dev.	0,41	0,45	0,42	0,43	0,46	0,47	0,48	0,45
Mean Diameter (µm) fibres l>20 µm	1,06	1,07	1,08	0,96	1,08	1,07	1,11	1,06
Std. Dev.	0,39	0,46	0,43	0,39	0,46	0,32	0,44	0,42
Mean Diameter (µm) WHO-fibres	1,06	1,13	1,09	1,07	1,09	1,17	1,15	1,11
Std. Dev.	0,45	0,47	0,44	0,45	0,46	0,47	0,48	0,46
Mean Length (µm) total fibres	7,22	9,45	8,46	8,52	8,95	7,77	9,32	8,41
Std. Dev.	5,86	7,24	6,84	6,60	6,99	5,88	7,37	6,67
Mean Length (µm) fibres l>20 µm	26,87	27,07	28,80	26,48	26,04	27,44	27,00	26,99
Std. Dev.	6,77	6,06	7,77	6,03	5,56	8,30	6,39	6,57
Mean Length (µm) WHO-fibres	10,34	12,36	11,46	11,27	11,88	10,47	12,28	11,37
Std. Dev.	6,38	7,22	7,18	6,84	7,07	6,14	7,58	6,92
GMD (µm) total fibres	0,84	0,90	0,88	0,87	0,88	0,93	0,96	0,89
Std. Dev.	1,58	1,57	1,55	1,59	1,61	1,58	1,58	1,59
GMD (µm) fibres l>20 µm	0,99	0,98	1,00	0,89	0,99	1,03	1,03	0,99
Std. Dev.	1,45	1,52	1,46	1,50	1,51	1,34	1,48	1,48
GMD (µm) WHO-fibres	0,97	1,04	1,00	0,98	1,00	1,08	1,05	1,02
Std. Dev.	1,57	1,53	1,52	1,56	1,55	1,51	1,56	1,54
GML (µm) total fibres	5,74	7,36	6,67	6,78	6,94	6,29	7,27	6,62
Std. Dev.	1,91	2,02	1,95	1,93	2,03	1,88	1,99	1,96
GML (µm)fibres l>20 µm	26,16	26,49	27,90	25,91	25,56	26,59	26,37	26,33
Std. Dev.	1,25	1,22	1,28	1,22	1,21	1,27	1,23	1,24
GML (µm) WHO-fibres	9,06	10,76	9,98	9,79	10,29	9,30	10,56	9,90
Std. Dev.	1,61	1,66	1,63	1,65	1,67	1,58	1,70	1,65
Length weighted arthm. diameter (µm)	1,02	1,08	1,04	1,01	1,09	1,11	1,14	1,07
Length weighted geom. diameter (µm)	0,90	0,96	0,93	0,91	0,95	0,99	1,01	0,95
Mode diameter (µm)	1,3	0,9	1,0	1,1	1,1	1,3	1,0	1,0
Mode length (µm)	4,3	4,7	5,0	3,5	3,4	4,8	4,0	4,3
Median diameter (µm)	0,9	0,9	0,9	0,9	0,9	1,0	1,0	0,9
Median length (µm)	5,4	6,8	6,4	6,2	6,5	6,0	6,7	6,2
Aspect ratio	8,7	10,5	9,6	10,3	10,1	8,4	10,0	9,57
Total length (m)	251,8	241,4	178,1	185,7	220,4	232,4	184,7	213,5
fibre-mass in mg per lung (density 1,5 g/cm^3)	0,3	0,3	0,2	0,2	0,3	0,3	0,3	0,3
Number of Particles evaluated	102,5	102,5	100	102	102	101	101	711
Mean Number of Particles per lung lobes in millions	25,303	12,049	13,263	19,817	26,710	15,111	13,477	17,961
Number of particles/(fibres+particles) in %	42%	32%	39%	48%	52%	34%	40%	41%
<= 1µm particles per lung lobes in millions	0,987	0,235	0,133	0,583	3,142	0,299	0,133	0,788
> 1µm - <= 3µm particles per lung lobes in millions	22,217	10,051	10,080	16,126	20,687	12,343	11,342	14,692
> 3µm particles per lung lobes in millions	2,098	1,763	3,050	3,109	2,880	2,469	2,002	2,482

Table A3: Aerosol	Control	Carbon fibres
samples evaluated	5	5
Number of Fibres evaluated	2	3104,5
Number of total fibres per cm^3 air	0,014	1611,188
WHO Fibres per cm^3 air	0.000	1046,079
Number of WHO-fibres/total fibres in %	0%	65%
Number of WHO-fibres l>20µm per cm^3 air	0,000	88,631
Number of WHO-fibres l>20µm/WHO-fibres in %	N.A.	8%
Number of WHO-fibres l>40µm per cm^3 air	0,000	8,934
Number of WHO-fibres l>40µm/WHO-fibres in %	N.A.	0,9%
Number of fibres l >20 µm per cm^3 air	0,000	92,050
Number of fibres l>20 µm/total fibres in %	0%	6%
Number of fibres l >40 µm per cm^3 air	0,000	10,074
Number of fibres l>40 µm/total fibres in %	0,0%	0,6%
Number of fibres l 5 - 20 µm per cm^3 air	0,000	983,248
Number of fibres l <5 µm per cm^3 air	0,014	535,889
Diameter Range (µm)	0,90 - 1,2	0,10 - 5,3
Length Range (µm)	3,2 - 4,2	1,3 - 110,0
Mean Diameter (µm) total fibres	1,05	1,04
Std. Dev.	0,17	0,54
Mean Diameter (µm) fibres l>20 µm	N.A.	1,33
Std. Dev.	N.A.	0,77
Mean Diameter (µm) WHO-fibres	N.A.	1,17
Std. Dev.	N.A.	0,52
Mean Length (µm) total fibres	3,70	8,49
Std. Dev.	0,58	7,19
Mean Length (µm) fibres l>20 µm	N.A.	30,22
Std. Dev.	N.A.	11,78
Mean Length (µm) WHO-fibres	N.A.	10,95
Std. Dev.	N.A.	7,46
GMD (µm) total fibres	1,04	0,92
Std. Dev.	1,18	1,68
GMD (µm) fibres l>20 µm	N.A.	1,15
Std. Dev.	N.A.	1,72
GMD (µm) WHO-fibres	N.A.	1,05
Std. Dev.	N.A.	1,64
GML (µm) total fibres	3,67	6,74
Std. Dev.	1,17	1,92
GML (µm)fibres l>20 µm	N.A.	28,72
Std. Dev.	N.A.	1,34
GML (µm) WHO-fibres	N.A.	9,53
Std. Dev.	N.A.	1,62
Length weighted arthm. diameter (µm)	1,07	1,19
Length weighted geom. diameter (µm)	1,05	0,99
Mode diameter (µm)	0,9	1,2
Mode length (µm)	3,2	4,7
Median diameter (µm)	1,1	1,0
Median length (µm)	3,7	6,4
Aspect ratio	3,5	9,5
Total length (m) per m^3 air	0,1	13679,2
fibre-mass in mg per m^3 air (density 1,5 g/cm^3)	0,000	22,461
Number of Particles evaluated	3	509
Mean Number of Particles per cm^3 air	0,020	888,219
Number of particles/(fibres+particles) in %	60%	36%
<= 1µm particles per cm^3 air	0,000	46,101
> 1µm - <= 3µm particles per cm^3 air	0,020	697,788
> 3µm particles per cm^3 air	0,000	144,329

Table A4: Air Control			
	day 1	day 29	day 92
samples evaluated	5	5	5
Number of Fibres evaluated	4	3,5	8
Number of total fibres per lung lobes in millions	0,041	0,036	0,082
WHO Fibres per lung lobes in millions	0,020	0,036	0,031
Number of WHO-fibres/total fibres in %	50%	100%	38%
Number of WHO-fibres l>20µm per lung lobes in	0,000	0,000	0,000
Number of WHO-fibres l>20µm/WHO-fibres in %	0,0%	0,0%	0,0%
Number of WHO-fibres l>40µm per lung lobes in	0,000	0,000	0,000
Number of WHO-fibres l>40µm/WHO-fibres in %	0,0%	0,0%	0,0%
Number of fibres l >20 µm per lung lobes in millions	0,000	0,000	0,000
Number of fibres l>20 µm/total fibres in %	0,0%	0,0%	0,0%
Number of fibres l >40 µm per lung lobes in millions	0,000	0,000	0,000
Number of fibres l>40 µm/total fibres in %	0,0%	0,0%	0,0%
Number of fibres l 5 - 20 µm per lung lobes in millions	0,020	0,036	0,031
Number of fibres l <5 µm per lung lobes in millions	0,020	0,000	0,051
Diameter Range (µm)	0,57 - 2,1	1,00 - 1,5	0,51 - 2,6
Length Range (µm)	4,0 - 8,1	5,1 - 11,6	2,3 - 19,2
Mean Diameter (µm) total fibres	1,34	1,33	0,92
Std. Dev.	0,81	0,23	0,67
Mean Diameter (µm) fibres l>20 µm	N.A.	N.A.	N.A.
Std. Dev.	N.A.	N.A.	N.A.
Mean Diameter (µm) WHO-fibres	2,10	1,33	1,47
Std. Dev.	0,00	0,23	0,88
Mean Length (µm) total fibres	5,78	7,63	5,60
Std. Dev.	1,86	3,04	5,48
Mean Length (µm) fibres l>20 µm	N.A.	N.A.	N.A.
Std. Dev.	N.A.	N.A.	N.A.
Mean Length (µm) WHO-fibres	7,45	7,63	10,13
Std. Dev.	0,75	3,04	7,02
GMD (µm) total fibres	1,11	1,31	0,79
Std. Dev.	1,98	1,21	1,66
GMD (µm) fibres l>20 µm	N.A.	N.A.	N.A.
Std. Dev.	N.A.	N.A.	N.A.
GMD (µm) WHO-fibres	2,10	1,31	1,28
Std. Dev.	1,00	1,21	1,75
GML (µm) total fibres	5,52	7,15	4,22
Std. Dev.	1,38	1,47	2,01
GML (µm)fibres l>20 µm	N.A.	N.A.	N.A.
Std. Dev.	N.A.	N.A.	N.A.
GML (µm) WHO-fibres	7,42	7,15	8,44
Std. Dev.	1,11	1,47	1,89
Length weighted arthm. diameter (µm)	1,56	1,38	1,53
Length weighted geom. diameter (µm)	1,24	1,33	0,98
Mode diameter (µm)	2,1	1,5	0,6
Mode length (µm)	4,2	11,6	2,4
Median diameter (µm)	1,4	1,4	0,6
Median length (µm)	5,5	5,5	3,7
Aspect ratio	5,3	5,7	5,56
Total length (m)	0,2	0,3	0,5
fibre-mass in mg per lung (density 1,5 g/cm^3)	0,0	0,0	0,0
Number of Particles evaluated	7	7	9
Mean Number of Particles per lung lobes in millions	0,071	0,071	0,092
Number of particles/(fibres+particles) in %	64%	67%	53%
<= 1µm particles per lung lobes in millions	0,000	0,000	0,000
> 1µm - <= 3µm particles per lung lobes in millions	0,031	0,020	0,020
> 3µm particles per lung lobes in millions	0,041	0,051	0,071

Table A5: Carbon fibres					
	day 1	day 3	day 15	day 29	day 92
samples evaluated	7	7	7	7	7
Number of Fibres evaluated	4585	4820,5	4592	4527	3497,5
Number of total fibres per lung lobes in millions	83,858	82,122	61,764	53,500	25,378
WHO Fibres per lung lobes in millions	51,676	48,074	37,825	33,259	15,705
Number of WHO-fibres/total fibres in %	62%	59%	61%	62%	62%
Number of WHO-fibres l>20µm per lung lobes in	3,023	2,940	2,332	2,191	1,734
Number of WHO-fibres l>20µm/WHO-fibres in %	6%	6%	6%	7%	11%
Number of WHO-fibres l>40µm per lung lobes in	0,136	0,117	0,160	0,079	0,073
Number of WHO-fibres l>40µm/WHO-fibres in %	0,3%	0,2%	0,4%	0,2%	0,5%
Number of fibres l >20 µm per lung lobes in millions	3,049	2,940	2,332	2,191	1,734
Number of fibres l>20 µm/total fibres in %	4%	4%	4%	4%	7%
Number of fibres l >40 µm per lung lobes in millions	0,136	0,117	0,160	0,079	0,073
Number of fibres l>40 µm/total fibres in %	0,2%	0,1%	0,3%	0,1%	0,3%
Number of fibres l 5 - 20 µm per lung lobes in millions	50,133	46,599	36,716	32,377	14,450
Number of fibres l <5 µm per lung lobes in millions	30,676	32,583	22,716	18,932	9,195
Diameter Range (µm)	0,20 - 3,5	0,12 - 3,4	0,10 - 4,5	0,10 - 3,5	0,10 - 3,6
Length Range (µm)	1,3 - 65,0	1,0 - 55,0	1,3 - 75,0	1,0 - 70,0	1,2 - 70,0
Mean Diameter (µm) total fibres	0,96	0,96	0,99	0,98	0,99
Std. Dev.	0,44	0,44	0,46	0,45	0,45
Mean Diameter (µm) fibres l>20 µm	1,05	1,07	1,13	1,07	1,06
Std. Dev.	0,47	0,44	0,46	0,43	0,42
Mean Diameter (µm) WHO-fibres	1,08	1,10	1,12	1,11	1,11
Std. Dev.	0,46	0,47	0,48	0,47	0,46
Mean Length (µm) total fibres	7,60	7,34	7,66	7,80	8,41
Std. Dev.	5,47	5,34	5,67	5,68	6,67
Mean Length (µm) fibres l>20 µm	26,62	26,15	27,32	26,73	26,99
Std. Dev.	6,98	5,95	7,36	7,07	6,57
Mean Length (µm) WHO-fibres	10,10	10,03	10,23	10,31	11,37
Std. Dev.	5,58	5,52	5,87	5,86	6,92
GMD (µm) total fibres	0,87	0,86	0,89	0,88	0,89
Std. Dev.	1,59	1,61	1,60	1,59	1,59
GMD (µm) fibres l>20 µm	0,96	0,98	1,04	0,99	0,99
Std. Dev.	1,51	1,53	1,51	1,49	1,48
GMD (µm) WHO-fibres	0,98	1,00	1,02	1,01	1,02
Std. Dev.	1,57	1,56	1,57	1,56	1,54
GML (µm) total fibres	6,24	6,02	6,26	6,39	6,62
Std. Dev.	1,85	1,84	1,85	1,85	1,96
GML (µm)fibres l>20 µm	25,92	25,60	26,53	26,02	26,33
Std. Dev.	1,24	1,22	1,26	1,24	1,24
GML (µm) WHO-fibres	9,08	9,00	9,14	9,19	9,90
Std. Dev.	1,54	1,55	1,56	1,57	1,65
Length weighted arthm. diameter (µm)	1,04	1,05	1,08	1,07	1,07
Length weighted geom. diameter (µm)	0,92	0,92	0,94	0,94	0,95
Mode diameter (µm)	1,0	1,2	1,0	1,0	1,0
Mode length (µm)	4,0	4,7	4,5	4,0	4,3
Median diameter (µm)	0,9	0,9	0,9	0,9	0,9
Median length (µm)	6,1	5,8	6,0	6,0	6,2
Aspect ratio	9,0	8,7	8,8	9,0	9,57
Total length (m)	637,3	603,1	473,3	417,4	213,5
fibre-mass in mg per lung (density 1,5 g/cm^3)	0,8	0,7	0,6	0,5	0,3
Number of Particles evaluated	722,5	725	713,5	711,5	711
Mean Number of Particles per lung lobes in millions	42,148	55,080	40,861	36,714	17,961
Number of particles/(fibres+particles) in %	33%	40%	40%	41%	41%
<= 1µm particles per lung lobes in millions	1,601	3,184	2,041	1,867	0,788
> 1µm - <= 3µm particles per lung lobes in millions	35,567	46,524	34,189	29,206	14,692
> 3µm particles per lung lobes in millions	4,980	5,372	4,631	5,641	2,482

RCC STUDY NUMBER 801314

CARBON FIBRE

3-MONTH BIOPERSISTENCE INHALATION STUDY IN THE RAT

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FIRST AMENDMENT TO REPORT

RCC

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Date: 16-JAN-2003

MANAGEMENT

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STATEMENT

RCC Study Number	801314
Test Item	CARBON FIBRE
Study Director	Paul A. Smith
Title	CARBON FIBRE: 3-Month Biopersistence Inhalation Study in the Rat

This amendment to report was audited by the Quality Assurance Unit. The date is given below.

The general facilities and activities are inspected periodically and the results are reported to the responsible person and the management.

Date and Type of QAU Inspection	Date of Report to the Study Director and to Management
30-OCT-2002 Amendment to report	30-OCT-2002

This statement also confirms that this amendment to report reflects the raw data.

Quality Assurance Th. Frei

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Date: 16 January 2003

A

CONCERNING: **5.1 ANIMAL DATA**

PAGE: 10

PRESENT: Macroscopic findings at the 1 and 3-day sacrifice time-points indicated a minor, acute response to inhalation of a particulate material. Increased lung weights at the 29 and 92-day sacrifice time-points were observed, but it is not clear as to whether these were treatment related.

NEW: Macroscopic findings at the 1 and 3-day sacrifice time-points indicated a minor, acute response to inhalation of a particulate material. Increased lung weights at the 29 and 92-day sacrifice time-points were observed, attaining statistical significance on day 92, but it is not clear as to whether these were treatment related.

REASON FOR THE ALTERATION: Clarification of significance.

B

CONCERNING: **7.8 PATHOLOGY - Organ Sampling**

PAGE: 17

PRESENT: All freeze-dried lungs were chemically digested according to a method established at RCC and sent to GSA for lung burden investigations. The method comprised digesting the lungs in a heated acid mixture for a set duration followed by similar treatment with an oxidising agent and then vacuum filtering to trap the organic fibres on polycarbonate filters.

NEW: All freeze-dried lungs were chemically digested according to a method established at RCC and sent to GSA for lung burden investigations. The method comprised digesting the lungs in an acid mixture for two hours at 135 to 140°C, followed by similar treatment twice for 30 minutes with an oxidising agent and a cooling time between both digestions. After cooling, vacuum filtering was performed to trap the organic fibres on polycarbonate filters, remaining fibres were collected by rinsing twice the material with 1 ml Methanol and filtering the liquid on the same polycarbonate filter.

This method was developed and carefully validated at RCC. During pretests, lungs were spiked with a known amount of test item in order to monitor the recovery of unbroken fibres. The digestion method showed that the lung tissue was well eliminated without changing the fibre morphology and that recovery of the carbon fibre was good.

REASON FOR THE ALTERATION: Specification of the digestion procedure on request by the Sponsor.

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Note: Amended pages are attached to this amendment

5 SUMMARY

The purpose of this study was to assess the in-vivo pulmonary biopersistence of inhaled fibrous and non-fibrous particles in the rat following repeated inhalation exposure.

Laboratory rats were randomly assigned to one negative control group and to one group exposed to the test item, Carbon Fibre. The animals were exposed to filtered air (negative control) or to well characterised fibres which were sized to be rat respirable using a flow past, nose only exposure system. In the fibre-exposed group, the concentration was targeted to 15 mg/m^3 if technically feasible. The achieved concentration was 16 mg/m^3. The rats were exposed for five consecutive days, 6 hours per day, with a subsequent non-exposure period. In the control and in the fibre-exposed groups, rats were allocated to sub-groups of 5 and 7 animals respectively and sacrificed at 1 day, 3 days, 15 days, 29 days and 92 days.

For each of the sacrifice time-points the lung burden was determined by suitable extraction and measurement methods. Evaluation included counting of the number of fibres and particles in the lungs and characterisation of the fibres by bivariate analysis of diameter and length.

5.1 ANIMAL DATA

The in-life data recorded during the study - mortality, clinical signs and body weights - gave no indications of fibre-related findings.

Macroscopic findings at the 1 and 3-day sacrifice time-points indicated a minor, acute response to inhalation of a particulate material. Increased lung weights at the 29 and 92-day sacrifice time-points were observed, attaining statistical significance on day 92, but it is not clear as to whether these were treatment related.

5.2 LUNG BURDEN DATA

The main results of lung burden analyses by SEM performed at GSA are summarised below. Details are presented in Attachment 2.

Group 2, CARBON FIBRE						
time point	**1 day**	**3 days**	**15 days**	**29 days**	**92 days**	**T ½ days**
FIBRE NUMBER (10^6 / lung)						
Total fibres/lung	83.858	82.122	61.764	53.500	25.378	45.13s
WHO fibres/lung	51.676	48.074	37.825	33.259	15.705	50.36s
Fibres/lung < 5 µm	30.676	32.583	22.716	18.932	9.195	47.14d
Fibres/lung 5-20 µm	50.133	46.599	36.716	32.377	14.450	46.10s
Fibres/lung > 20 µm	3.049	2.940	2.332	2.191	1.734**	99.86s
Total length of fibres/lung (m)	637.3	603.1	473.3	417.4	213.5	49.82s
Non-fibre particles	42.148	55.080	40.861	36.714	17.961	---
N=	6*	7	7	7	7	

* Animal 22 was excluded because the value of the total fibre length (m), and the total number of fibres per lung, differed from the average value of all animals evaluated at the same time-point by more that two times the value of the standard deviation.

** The number of fibres > 20µm was statistically significantly lower ($p < 0.01$) on day 92 compared with day 1.

s Single exponential

d double exponential (weighted value)

Organ Sampling

All action was taken to avoid contamination of the dissected specimen by fibres from the fur or deposited on dissecting instruments. Special care was taken to avoid inter-group contamination. During each necropsy session, air control animals were dissected first.

For each animal the following procedure was followed:

- **The lungs and trachea** (sectioned below the larynx) were removed with the attached mediastinal tissue. The mediastinal tissue containing the mediastinal lymph nodes was resected and immediately deep-frozen on dry ice and then stored at -20°C or below, at RCC for possible investigations prescribed by the Sponsor under separate contractual agreement. However, if not used, storage of these specimens ends by the time of submission of the final report. The remaining tissue/organs: lower half of the trachea, main stem bronchi and lungs were weighed (recorded as "lung weight").
- **The trachea and the main stem bronchi** down to the limit of the lung lobes were resected in one piece, weighed (recorded as "trachea"), individually inserted into plastic bags appropriately labelled and immediately deep-frozen on dry ice and then stored at -20°C or below, at RCC for possible investigations prescribed by the Sponsor under separate contractual agreement. However, if not used, storage of these specimens ends by the time of submission of the final report.
- **All lung lobes** were weighed together (recorded as "all lung lobes"), immediately deep-frozen on dry ice and forwarded (deep-frozen) to RCC ECP (attn: M. Kern). The lungs were freeze dried, weighed (recorded as "dry lung weight"), inserted into a plastic bag appropriately labelled, then stored in a desiccator at room temperature until further processing.

All freeze-dried lungs were chemically digested according to a method established at RCC and sent to GSA for lung burden investigations. The method comprised digesting the lungs in an acid mixture for two hours at 135 to 140°C, followed by similar treatment twice for 30 minutes with an oxidising agent and a cooling time between both digestions. After cooling, vacuum filtering was performed to trap the organic fibres on polycarbonate filters, remaining fibres were collected by rinsing twice the material with 1 ml Methanol and filtering the liquid on the same polycarbonate filter.

This method was developed and carefully validated at RCC. During pretests, lungs were spiked with a known amount of test item in order to monitor the recovery of unbroken fibres. The digestion method showed that the lung tissue was well eliminated without changing the fibre morphology and that recovery of the carbon fibre was good.